University of Alberta

The Effects of Rowing Exercise and Carbohydrate Supplementation on the Immune System.

by

Christopher Michael Sellar

A thesis submitted to the Faculty of Graduate Studies and Research
in partial fulfillment of the requirements for the degree of:
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Date: January 28TH, 2003

To Mom & Dad:
For your endless love and support that
has helped me get to where I am today.

ABSTRACT

The purpose of this study was to investigate the transient post exercise alterations in immune function following 1 hour of rowing exercise. Furthermore, the effect of carbohydrate supplementation on these changes in immune function following exercise was determined. One hour of rowing exercise at 72% of VO_2max provided sufficient stimulus to alter post exercise immune function when compared to resting values. Carbohydrate supplementation before, during, and after the rowing exercise was only able to attenuate the increase in circulating lymphocytes five minutes after exercise when compared to a placebo group. All other blood, immune, and hormonal measures were not altered by carbohydrate ingestion. The results of this investigation have shown that one hour of rowing exercise provides a sufficient stimulus to alter the acute immune response to exercise. Also, carbohydrate supplementation was found to have little effect on immune function following exercise of this type, intensity, and duration.

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LIST OF NOMENCLATURE & ABBREVIATIONS

WBC	White Blood Cell
RBC	Red Blood Cell
NK Cell	Natural Killer Cell
NKCA	Natural Killer Cell Cytotoxic Activity
CD	Cluster of Differentiation
$CD3^+/4^+$	Helper T Cell (T_H Cell)
$CD3^+/8^+$	Cytotoxic or Suppresor T Cell (T_C or T_S Cell)
$CD14^+$	Monocytes
$CD16^+/56^+$	Natural Killer Cell
$CD20^+$	B Cell
$CD25^+$	IL-2 receptor α subunit
mAb	Monoclonal Anitbody
IL-2	Interleukin-2
IFNγ	Interferon-gamma(γ)
URTI	Upper Respiratory Tract Infection
HPA Axis	Hypothalamus-Pituitary-Adrenal Axis
ACTH	Adrenocorticotrophic Hormone
CHO	Carbohydrate
PLA	Placebo
GL	Glycemic Load
HR	Heart Rate
1RM/mRM	One/Multiple Repetition Maximum
VO_2max	Maximal Oxygen Consumption
VT	Ventilatory Threshold
RER	Respiratory Exchange Ratio

CHAPTER 1

INTRODUCTION

1.1 Introduction

Rowing exercise involves a very large percentage of the body's muscle mass, as well as the contribution of all three of the body's energy systems (Secher, 1993). As with other full-body intense exercise, rowing causes alterations in certain aspects of the immune response (Shephard, 1998) leaving a possibility of an "open window" to infection (Nieman, 2000). In addition, rowers are known to experience high levels of stress hormones (Nieman et al, 1999). Gleeson and Bishop (2000) have suggested that high levels of stress hormones are responsible for the observed changes in immune function following a bout of exercise. Furthermore, when the duration or intensity (or a combination of both) of an exercise bout is increased, the extent of the effect on the immune system is increased as well (Nieman, 2000). Recent research has suggested that supplementation of carbohydrate prior to, during, and after exercise may attenuate immune suppression following a bout of exercise (Gleeson and Bishop, 2000).

1.2 Purpose and Hypothesis

The purpose of this study was to investigate the effects of carbohydrate supplementation on the acute immune response to a 1-hour moderate intensity endurance row. It was hypothesized that 1 hour of rowing exercise would alter immune function following exercise. Furthermore, it was hypothesized that rowers who consume a carbohydrate beverage prior to, during, and following the 1-hour endurance row, will see an attenuated immune response following the rowing exercise.

1.3 Significance

Current research involving rowers has only investigated the immune response to short duration, high intensity exercise (Penkman, 2000, Nielsen, 1996) or to long duration, low intensity exercise (Nieman et al, 1999). This study attempts to show the immune response to moderate intensity, endurance exercise, which is similar to a training session that would be performed by a rower in off-season training. The documented acute immune response to this type of exercise, in combination with carbohydrate supplementation, would provide the rational to establish in guidelines and precautions to minimize any health risks that a rower may experience following a training session. Furthermore, this information could be extended to individuals who complete similar training sessions for other sports.

1.4 Delimitations

For the completion of this study, twenty-two male subjects performed a 1-hour endurance row for maximum distance and gave three blood samples: one fasted, rested sample before the rowing bout, and two samples at 5 and 60 minutes after the completion of the row. All blood samples were analyzed for the following: blood glucose, hematocrit, hemoglobin, cortisol, adrenocorticotrophic hormone (ACTH), plasma cytokine proliferation (IL-2, IFNγ), natural killer cell activity (NKCA), lymphocyte phenotyping (immunofluorescence), total circulating leukocytes, and the circulating number and percentage of lymphocytes, neutrophils, monocytes. Heart rate, power output, 500m split-time, and stroke rate were recorded throughout the 1-hour period and the final distance was recorded upon completion of the test.

In addition, all subjects completed a 2000-metre simulated rowing race at least three days prior to the 1-hour row. This allowed the subjects to be matched on 2000-metre time and randomly assigned to either the carbohydrate or placebo group. Heart rate, average power output, 500m split-times and stroke rate were recorded during the 2000-metre test. After the 1-hour row had been completed, subjects completed a combined ventilatory threshold/maximum oxygen consumption test (VT/VO_2max), and a multiple repetition maximum (mRM) strength test on separate days. Oxygen consumption, heart rate, power output, and stroke rate were measured throughout the VT/VO_2max test. The mRM strength testing was used to determine the maximum weight (kg) that can be lifted for 8 to 10 repetitions for both the bench and leg press. The result of the mRM test was used to estimate the 1-RM for both the subject's bench press and leg press.

1.5 Limitations

Due to the complexity of the immune system itself as well as its interactions with the other systems of the body, the measures taken for this study will be used as general indications of overall functional changes in the immune system.

Also, due to the nature of the 1-hour row for maximum distance, it was not possible to ensure that all subjects were performing at the same relative intensity. Since both the immune response (Nieman, 2000) and rates of carbohydrate depletion (Lamb and Murray, 1988) are directly related to the workload, this could cause variation in both the immune response to exercise and the effects of carbohydrate supplementation on the immune system of the subjects.

As well, subjects were required to follow a modified diet for the 3-day period before and fast from 10:00pm the night before their 1-hour row. If subjects did not follow the diets and fast as instructed, the substrate availability would be affected. This could possibly interfere with the supplementation procedure and thus affect any differences in the immune and hormonal responses to exercise between the carbohydrate and placebo groups.

The subject population for this study was limited to male subjects only. Due to the interactions between the endocrine and the immune systems (Weicker and Werle, 1991), all female subjects would have to be tested on the same day of their menstrual cycle. Therefore, the results of this study, obtained from males, may not be applicable to a female rowing population.

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CHAPTER 2

REVIEW OF LITERATURE

In order to explore all topics relevant to the purpose of this study, the literature review will be presented in the following sections: Overview of the Immune System; Immune Cell Metabolism; Interaction between the Endocrine and Immune Systems; The Acute Immune Response to Endurance Exercise; Immunosuppression of the Athlete; Carbohydrate and Exercise; Carbohydrate, Exercise, and the Immune System; The Physiology of Rowing; and Rowing and the Immune System.

2.1 Overview of the Immune System

The immune system is a complex system capable of recognizing and defending the human body against foreign invaders from the environment (Mackinnon, 1992). It protects the body from a wide range of foreign invaders including viruses, bacteria, fungi, parasites, tumor growth, and allergens.

The immune system can be divided into two major systems or responses known as *innate* and *adaptive* immunity (Goldsby et al, 2000). Innate immunity is the natural response of the immune system, which is the first response to a foreign agent encountered by the body (Woods et al, 1999). The innate immune response includes physical and chemical barriers that prevent or make it impossible for foreign agents to enter the body, as well as cells that can recognize and kill some foreign cells in the body (Goldsby et al, 2000). Adaptive immunity, as the name suggests, is an adaptive response by the immune system to a pathogen. Adaptive immunity can be further divided into a humoral arm, which includes antibodies and a memory response, as well as cells that either stimulate other immune cells or directly kill the foreign agents (Mackinnon, 1999). Upon first

exposure, its response is not immediate, but after a few days, adaptive immunity is fully activated (Goldsby et al, 2000). Adaptive immunity involves a specific response to each particular invading substance, which is developed through prior exposure to the pathogen (Goldsby et al, 2000). The adaptive response creates a memory of each encounter with a foreign agent, allowing a quicker and more efficient response to any repeated exposure (Kuby, 1997). The specificity of the adaptive immune response makes it more effective, flexible, and complex (Mackinnon, 1999). While the two systems are quite different, both the innate and adaptive systems work together and are very important for the overall functioning of the body's immune system (Goldsby et al, 2000).

The immune system follows a generalized response each time it encounters an invading pathogen in the body (Mackinnon, 1999). If a foreign agent manages to penetrate the physical and chemical barriers protecting the body, the agent will be met and engulfed by a phagocyte (Mackinnon, 1999). The phagocyte will engulf the foreign cell and breakdown its proteins. These proteins, also known as antigens, are then expressed on the phagocyte's surface, which alerts and activates helper T cells (T_H) (Mackinnon, 1999). The $CD4^+$ T cells further activate other specialized immune cells. These include B cells, that create antibodies to the antigen expressed by the phagocyte, natural killer (NK) cells that kill infected cells, cytotoxic T cells (T_C) to kill invading and infected cells, and memory B and T cells that will respond during a subsequent infection by the same foreign agent (Goldsby et al, 2000). This response can usually destroy an invading pathogen within a few days to weeks, however, the body's defenses are some times ineffective or inappropriate and infection may persist (Mackinnon, 1999).

All cells of the immune system originate from the hematopoietic stem cells found in bone marrow (Mackinnon, 1999). The cells are termed *leukocytes* and are found in lymphoid organs and tissues throughout the body, as well as in lymph and blood circulation (Goldsby et al, 2000). Leukocytes are more commonly known as *white blood cells* when they are found in the blood. After they have originated in the bone marrow, the leukocytes undergo further maturation and differentiation (Mackinnon, 1999). The leukocytes that are found in circulation are further subdivided into three major categories: *granulocytes*, *monocytes*, and *lymphocytes*.

In humans, granulocytes make up approximately 50-70% of circulating leukocytes or 3-6 million cells per litre of blood (Kuby, 1997). The most abundant granulocytes are neutrophils, which account for 90% of all granulocytes (Mackinnon, 1999). Neutrophils are produced in the bone marrow and are released into the bone where they circulate for 7 to 10 days before migrating into tissue where they reside for another three days (Kuby, 1997). Neutrophils serve a role in the innate immune response through the phagocytosis of foreign agents (Kuby, 1997). Other granulocytes include eosinophils and basophils.

Monocytes make up about 1-6% (0.15-0.60 million cells per litre of blood) of the circulating leukocyte numbers (Mackinnon, 1999). Monocytes differentiate into macrophages when they enter tissue, and like granulocytes, they play a role in the innate immune response. Macrophages have a primary function of phagocytosis (Mackinnon, 1999).

Lymphocytes account for approximately 20-40% of leukocytes in the blood or about 1-2.5 million cells per litre of blood and 99% of cells in lymph circulation (Kuby,

1997). Around 1-2% of the total lymphocyte pool is circulating in the blood at any time with the remaining lymphocytes residing in lymphoid tissue (Mackinnon, 1999). Lymphocytes are further subdivided into three subsets: T cells, B cells, and natural killer cells. T cells make up 60-75% of all circulating lymphocytes (Mackinnon, 1999) and are further divided into $CD4^+$ T cells (helper T cells (T_H)) and $CD8^+$ T cells (cytotoxic T cells (T_C) and suppressor T cells (T_S)) (Goldsby et al, 2000). The ratio of $CD4^+$ to $CD8^+$ T cells is approximately 2:1 in human peripheral blood (Goldsby et al, 2000). $CD8^+$ T cells, when activated, acquire cytotoxic activity and destroy infected cells (Goldsby et al, 2000). The primary functions of $CD4^+$ T cells include cytokine secretion, and B and $CD8^+$ T cell activation (Goldsby et al, 2000). $CD4^+$ T cells initiate at least two distinct responses depending on the cytokines they release. The responses are designated as either a T_H1 response, which activates mainly $CD8^+$ T cells and macrophages, or a T_H2 response, which activates mainly B cells (Kuby, 1997). B cells account for 5-15% of circulating lymphocyte levels (Mackinnon, 1999) and their main functions are antibody production, memory, and cytokine production (Kuby, 1997). Natural killer (NK) cells make up the remaining 5-10% of circulating lymphocytes and carry out the functions of cytotoxicity (natural killer cell activity or NKCA) and cytokine production (Goldsby et al, 2000).

It is important to note that all of the circulating cell numbers given above are for resting levels only. Exercise can cause dramatic changes in the percentages and numbers of the various leukocytes and lymphocytes. These changes will be discussed later on in this review.

All of the above leukocytes display a particular arrangement of membrane proteins that can be identified by particular monoclonal antibodies (Goldsby et al, 2000). These proteins can be used to identify the different types of leukocytes at various stages of maturation and activation (Mackinnon, 1999). Specific monoclonal antibodies (mAbs) have been raised to specific cell proteins for the purpose of identifying and quantifying the particular cell or the proteins they express. All of the antibodies that react with a particular cell surface protein are grouped together as a cluster of differentiation (CD), and along with a number refer to a particular type of cell or the proteins they express (Mackinnon, 1999). Some of the more common CDs are: CD $16^+/56^+$ (natural killer cells), CD $3^+/4^+$ (T_H cells), $CD3^+/8^+$ (T_C and T_S cells) and $CD20^+$ (B cells) (Goldsby et al, 2000).

While this discussion has focused on the cells of the immune system, there are many soluble factors that play integral roles in the immune response. The major soluble factors of the immune system are cytokines (Mackinnon, 1999). Cytokines are polypeptides that primarily function as regulatory of cells, but may also be mediators of the inflammatory response after exercise (Mackinnon, 1999). Cytokines are involved in almost every aspect of the immune response, as they act on many different target cells (some of which are non-immune cells), with each cell responding in a different way (Goldsby et al, 2000). Cytokines may be produce in non-immune cells but the main producers appear to be T cells, B cells, and macrophages (Goldsby et al, 2000). Due to the wide range of origins and functions, cytokines have been divided into several classes, with each class having a wide range of functions (Mackinnon, 1999). The three major classes are *interleukins* (IL), *interferons* (IFN), and *tumor necrosis factor* (TNF). For the

purpose of the study, interleukin-2 (IL-2) and interferon-gamma (IFNγ) will now be explored further.

IL-2 is released by activated $CD4^+$ T cells and is one of the cytokines secreted as part of the T_H1 response described above (Goldsby et al, 2000). The target cells of IL-2 include antigen primed $CD4^+$ and $CD8^+$ T cells, T cell clones, and NK cells (Goldsby et al, 2000). The biological function of IL-2 includes inducing a proliferation response (of $CD4^+$ and $CD8^+$ T cells), supporting growth (T cell clones), and most importantly for this study, has been found to stimulate NK and cytotoxic activity (Goldsby et al, 2000).

Interferons are factors able to interfere with viral replication and the spread of viruses from infected to non-infected cells (Mackinnon, 1999). The interferon that will be measured in this study will be IFNγ. IFNγ is secreted by $CD8^+$ T cells, T_H1 cells, and NK cells (Goldsby et al, 2000). This particular cytokine acts on a number of cells and stimulates a wide variety of responses in the target cells. The target cells include uninfected cells, macrophages, proliferating B, T_H2, and inflammatory cells (Goldsby et al, 2000). The activities stimulated by IFNγ include inhibition of viral replication, enhanced macrophage activity, and inhibited growth of T_H2 cells (Goldsby et al, 2000).

It is important to note that the biological effects of cytokines have been determined in isolated sample of a single cytokine (Goldsby et al, 2000). However, in the body, cytokines never act in isolation, but interact with other cytokines and cells of the immune system when responding to a pathogen (Goldsby et al, 2000).

2.2 Immune Cell Metabolism

Like all cells in the body, immune cells require fuel for metabolism. The rate of metabolism in immune cells is very high (Calder, 1995). Glucose has been identified as

the primary energy source for many cells of the immune system including lymphocytes, neutrophils, and macrophages (Gleeson and Bishop, 2000, Blannin et al, 1998). For example, it has been determined that glucose catabolism contributes 54% of ATP generation in macrophages with glutamine account for the balance of ATP production (Newsholme et al, 1996). Furthermore, the activation and proliferation of these cells when exposed to a mitogen, is also heavily dependent on glucose above the physiologic range (Calder, 1995, Hume and Weidemann, 1997).

Research has also revealed that the large majority of glucose utilized by immune cells is not completely oxidized and converted to lactate, even under aerobic conditions (Ardawi and Newsholme, 1985). This suggests that energy generation is not the primary use of glucose (and high rates of glycolysis) by immune cells (Calder, 1995). It has been proposed that glucose is also important in the regulation of the rates of synthesis of important molecules at specific times in the cell cycle (Calder, 1995).

Gleeson and Bishop (2000) suggest that the metabolic demands of immune cells stresses the importance of adequate dietary carbohydrate in maintaining immunocompetence. This is further supported by Calder (1995), who found that lymphocytes and macrophages rely more heavily on exogeneous carbohydrate stores as a fuel source than on endogeneous stores. Therefore, both the overall nutritional status of an athlete, as well as their nutrition before, during, and after exercise, are very important in maintaining immune function and reducing the risk of illness.

2.3 Interaction between the Endocrine and Immune Systems

Prolonged, intense exercise is linked to a number of hormonal changes, with a number of these changes having detrimental effects on the cells of the immune system

and their function (Gleeson and Bishop, 2000). While some hormones (primarily insulin) serve an essential role in immune cell metabolism and antibody production (Weicker and Werle, 1991), many of the stress hormones (cortisol and adrenocorticotrophic (ACTH) hormone) have been linked to the alterations of immune function (Gleeson and Bishop, 2000, Suzuki et al, 1999).

The nature of rowing exercise causes high circulating levels of stress hormones in rowers during and after exercise (Nieman et al, 1999a). Any intense, prolonged exercise bout results in the stimulation of the Hypothalamus-Pituitary-Adrenal (HPA) Axis which leads to the subsequent release of adrenocorticotrophic hormone (ACTH), which further causes the release of cortisol (Gleeson and Bishop, 2000). These stress hormones are known to effect the proliferation of cytokines (Nieman, 1999a, Weicker and Werle, 1991) and the number and activation of macrophages (Woods, 2000, Cupps and Fauci, 1982), T cells (Weicker and Werle, 1991, Cupps and Fauci, 1982), B cells (Weicker and Werle, 1991, Cupps and Fauci, 1982), and NK cells (Jonsdottir, 2000, Weicker and Werle, 1991). More specifically, high levels of cortisol have been shown to inhibit the anti-inflammatory response and the release of IL-1 (Weicker and Werle, 1991). This, in turn reduces the formation and release of IL-2 by T_H1 cells, which further reduces lymphocyte proliferation, B and NK cell activation (Weicker and Werle, 1991). It also prevents IFNγ from activating monocytes/macrophages (Woods, 2000, Cupps and Fauci, 1982). Furthermore, cortisol has been shown to alter leukocyte liberation from bone marrow and the distribution of leukocytes throughout the body (Weicker and Werle, 1991).

2.4 The Acute Immune Response to Endurance Exercise

Research has found that the immune response to prolonged intense, exercise is similar to its response to other forms of physical stress such as surgery or trauma (Nehlsen-Cannarella, 1998). In the literature, a 1-hour row is categorized in the literature as prolonged (between 1 to 3 hours), intense exercise (Mackinnon, 1999, Shephard and Shek, 1999). Therefore, the following discussion will focus on the immune response to prolonged, intense exercise. It is important to note that the intensity of the rowing exercise used for this investigation is generally classified as moderate intensity exercise in the exercise literature (ACSM Guidelines). For the purposes of this study, the current research on the acute response to exercise of total leukocyte, lymphocyte, neutrophil, natural killer cell numbers and activity, and cytokines will be examined.

Leukocytes

The general consensus in current research is that leukocyte numbers increase approximately 200-300% from resting levels immediately following prolonged, intense exercise, and may remain elevated at these levels for two to six hours after exercise (Mackinnon, 1999). This increase in leukocyte levels during, immediately following, and during recovery is known as leukocytosis (Pedersen and Hoffman-Goetz, 2000a). A relationship between the extent of change and persistence of the leukocytosis, and the intensity and duration of the exercise has been shown (Nieman and Pedersen, 1999b), where exercise of higher intensity or longer periods (or both), have shown longer periods of leukocytosis. While the mechanisms of recruitment and clearance of leukoctyes before and after exercise are not entirely known, it has been suggested that stress hormones (both glucocorticoids and catecholamines), cytokines, and the general increase

in cardiac output seen with exercise are responsible for exercise induced leukocytosis (Mackinnon, 1999). The major locations of recruitment of leukocytes during exercise are secondary lymphoid organs (spleen and lymph nodes) and also from tissue in the lungs and liver (Mackinnon, 1999). It has also been suggested that leukocytes may be recruited from primary lymphoid tissue during high intensity, prolonged exercise (Mackinnon, 1999). The above mechanisms and locations of recruitment of leukocytes during exercise also apply to the other classes of leukocytes that will now be discussed below (lymphocytes, neutrophils, and NK cells).

Lymphocytes

Much like leukocytes, lymphocyte numbers increase dramatically during and immediately following exercise (Pedersen and Hoffman-Goetz, 2000a). This increase can be as much as 30-100% immediately following prolonged, intense exercise (Mackinnon, 1999). An increase in the circulating lymphocyte number is known as lymphocytosis (Mackinnon, 1999) or lymphopenia (Weicker and Werle, 2000). However, where as leukocyte numbers continued to rise during recovery from exercise, the number of circulating lymphocytes generally returns close to or even below the resting levels of lymphocytes (Pedersen and Hoffman-Goetz, 2000a). Circulating lymphocyte numbers have been found to decrease anywhere from 30 to 50% below resting levels at 1 to 3 hours following exercise (Mackinnon, 1999). However, lymphocyte numbers will gradually return to normal resting levels after this initial decrease during recovery, which can take as long as six hours post exercise (Mackinnon, 1999). The changes seen in circulating lymphocyte numbers during and following exercise are known as the biphasic response of lymphocytes (Mackinnon, 1999). Again,

the duration and intensity of the exercise will determine the magnitude of both the increase in leukocyte numbers immediately following exercise and the depression in leukocyte numbers during recovery from exercise (Nieman and Pedersen, 1999b).

Neutrophils

The response of the circulating neutrophil numbers will follow a similar pattern to leukocyte levels during, immediately following, and in recovery from exercise (Pedersen and Hoffman-Goetz, 2000a). Neutrophil numbers have been found to increase as much as 300% during and immediately following prolonged exercise (Mackinnon, 1999). This response immediately following exercise is known as *neutropenia* (Kuby, 1997). These numbers remain elevated or may even increase further (300-400%) for to 2 to 6 hours post exercise (Mackinnon, 1999). This large increase in neutrophil numbers is much greater than the increase in leukocyte numbers following exercise, resulting in an increase in the neutrophil to lymphocyte ratio (Mackinnon, 1999). Nieman et al (1995) have suggested that this ratio can be used as an index of physiologic stress to the immune system as it is often correlated with cortisol levels. Again, the extent of changes in circulating neutrophil levels both during and after exercise is directly related to the intensity and duration of the exercise (Mackinnon, 1999).

Natural Killer Cells

Circulating natural killer cell numbers begin to increase early during exercise and continue to rise immediately following exercise (Nieman et al, 1997c). The range of this increase has been found to be between 300 to 500% immediately following prolonged, intense exercise (Shephard and Shek, 1999). Following exercise, circulating natural killer cell numbers have been found to drop up to 30-60% below normal, resting levels

(Mackinnon, 1999). This depression of natural killer cell numbers usually occurs between 1 to 2 hours following exercise and has been found to persist anywhere from 21 hours to 7 days following prolonged exercise (Mackinnon, 1999).

In addition, the cytotoxic activity of natural killer (or natural killer cell activity) cells is also affected by exercise. Most current research agrees that natural killer cell activity (NKCA) increases during exercise and remains elevated immediately following exercise regardless of the intensity or duration of the activity (Mackinnon, 1999). However, like other changes in circulating immune cell numbers during recovery, the intensity and duration of the exercise bout affect NKCA (Nieman and Pedersen, 2002 and 1999b). During and immediately following prolonged, intense exercise NKCA has been found to increase as much as 200% above resting levels (Shephard and Shek, 1999). However, during recovery, NKCA is suppressed for 1 to 2 hours following exercise, and may persist for hours or even days (Mackinnon, 1999). When NKCA has been expressed on a per cell basis in lytic units, post exercise values have not been statistically different than values at rest (Shephard and Shek, 1999, Nieman et al, 1997).

Cytokines

Any exercise that causes muscle damage, either strength training or intense, endurance activity will elicit an inflammatory response. Due to the large muscle mass and high intensities involved, rowing exercise will result in an inflammatory response (Nieman et al, 1999a). The inflammatory response involves a complex and dependent release of various cytokines, including IL-1, TNFα, IL-6, IL-1ra and stress hormones (ACTH and cortisol) (Nehlsen-Cannarella et al, 1997). The muscle damage will also directly result in activation of the Hypothalamic-pituitary-adrenal (HPA) axis and the

subsequent release of ACTH (Nehlsen-Cannarella et al, 1997). ACTH then releases stress hormones including cortisol, which has been linked to alterations in immune function (Gleeson and Bishop, 2000).

Of more importance to this study, the exercise response of IL-2 and IFNγ will be explored. In vitro production of IL-2 by phytothemagglutinin (PHA) stimulation of isolated lymphocytes has been found to be 20 to 80% lower immediately following exercise (Mackinnon, 1999, Weinstock et al, 1997, Rhind et al, 1996, Shephard et al, 1994) and remained significantly lower (30-40%) an hour after recovery. This decrease in IL-2 production was partially attributed to a decrease in $CD4^+$ cells, suggesting fewer T cells producing IL-2 (Mackinnon, 1999). An increase of IL-2 receptor α subunit ($CD25^+$) has also been found during and after exercise (Shephard et al, 1994). This increased expression of IL-2 receptor caused by exercise may be due to increased activation of cells (T and NK cells), increased NK cell recruitment into circulation, or enhanced clearance of IL-2, all of which may be responsible for the decrease IL-2 concentrations following exercise (Mackinnon, 1999).

Both IFNγ concentration in plasma and in vitro production of IFNγ have been found to remain unchanged during and following exercise when compared to resting values (Haahr et al, 1991). It is known that IFNγ is degraded rapidly in plasma following its release, which may be the reason that no change in IFNγ concentrations are found following exercise in many studies (Mackinnon, 1999). In contrast, the in vitro production of IFNγ was found to be significantly depressed when compared to rest after strenuous exercise (Northoff et al, 1997). Furthermore, the IFNγ concentration was

found to be elevated in urine samples immediately following exercise and returned to pre-exercise values five hours after exercise (Northoff et al, 1994).

2.5 Immunosuppression of the Athlete

From the current research base in the field of exercise immunology, it is possible to generalize that the effect of exercise on the immune system and its function is very dependent on both the duration and intensity of the exercise (Nieman, 2000c). A proposed "J-shaped" relationship has been put forward in order to describe the relationship between these variables. This model suggests that moderate exercise would provide the lowest risk of infection (Smith, 1995). This has been widely accepted as moderate exercise has been used in the prevention and treatment of illness and diseases that involve the immune system, including tumors, cancers, and even HIV (Mackinnon, 1999 and 1994). This model also suggests that individuals who are inactive and those who exercise at high intensities may be at a higher risk of becoming infected. The J-shape also suggest that athletes who train and participate in activities of very high intensities for prolonged time periods with inadequate recovery between training/activity periods would be at the highest risk of infection when compared to all other individuals in the population (Shephard, 1997).

While exercise can have beneficial effects on immune function, it is also possible that exercise may have negative effects on an individual's immune system. While methodology among current research makes it difficult to compare the resulting experimental evidence, there are some generalizations that have been made to characterize the relationship between athletes and risk of infection (Smith, 1995). It has been generally accepted that athletes, especially those who train and participate at high

intensities, seem to be more at risk of contracting illnesses (Nieman, 1998c and 1996, & Peters 1997), even though athletes are not clinically immunodeficient (Mackinnon, 1998). In the literature, the illness that is most often discussed is Upper Respiratory Tract Infection, or URTI (Nieman, 2000b). While studies have shown an increased rate of URTI in endurance athletes when compared to matched controls, a direct cause and effect relationship has not been established (Nieman, 1997b & 1994). Instead, the "Open Window" theory has been the suggested cause of the increased rate of infection following intense training or competition (Nieman and Pedersen, 1999b, Nieman, 1999c). This "Open Window" period occurs due to periods of transient, altered immune function following a period of intense physical exertion (Pedersen and Rohde, 1996). A number of exercise altered immune parameters that may lead to this "open window" have been suggested, including depressed NK cell numbers and activity, reduced lymphocyte numbers and proliferation, reduced neutrophil activity, and reduced cytokine production (Mackinnon, 1997). It has been suggested that this "window" may remain open anywhere from 3 hours to 7 days post-exercise, allowing an infectious agent to evade the innate immune defenses of the body (Nieman and Pedersen, 1999b).

2.6 Carbohydrates and Exercise

It is generally accepted that athletes require adequate carbohydrate available for the maintenance of training and performance. It is recommended that athletes' diets contain a minimum of 50% carbohydrate and a maximum of 60 to 70% for endurance athletes (Gleeson and Bishop, 2000, Coyle, 1988). It is also essential to ensure proper nutrition to maintain carbohydrate levels before, during, and after exercise of high intensity and long duration to maintain carbohydrate stores in the body. Reduced levels

of carbohydrate during and after exercise have been linked to higher levels of stress hormones, as well as greater perturbation of the immune system (Gleeson and Bishop, 2000). Fatigue, leading to reduced performance, is also a concern if muscle glycogen stores become depleted (Lamb and Murray, 1988).

Prior to exercise, it is recommended that an individual consumes 1 g of carbohydrate/kg of body weight between 45 to 60 minutes before beginning the exercise (Sherman, 1989). This practice has been demonstrated to increase both liver and muscle glycogen stores, which has the capacity to increase exercise performance (Febbraio et al, 2000b).

During exercise, ingestion of carbohydrate in solution provides a dual purpose. Intake of carbohydrate during exercise has been shown to prevent hypoglycemia later in exercise and improve performance (Febbraio et al, 2000b), in addition to maintaining hydration status (Coyle, 1994, and Coleman, 1988). Maintenance of hydration status has been found to reduce body temperature, heart rate, and perceived exertion during exercise (Coyle, 1994). The optimal range of carbohydrate concentration has been determined to be between 6 to 8% (Coyle, 1994, Gisolfi and Duchman, 1992), with any concentration of greater than 10% being detrimental, causing impaired gastric emptying and often associated with abdominal cramps, nausea, and diarrhea (Coleman, 1988). Also, recommendations suggest that an athlete drinks 3 ml/kg of body weight every 15 minutes in order to remain hydrated (Maughan and Rehrer, 1993). The type of carbohydrate must also be considered as glucose, sucrose, and glucose polymers stimulate fluid absorption in the small intestine, where as fructose impairs gastric emptying (Coleman, 1988). Furthermore, it has been recommended that carbohydrate solutions consumed during

exercise should include 10 to 20 mEq of both Na^+ and Cl^- to enhance carbohydrate absorption (Gisolfi and Duchman, 1992).

Following exercise, carbohydrate ingestion in solution again serves a dual purpose. The carbohydrate will restore the body's deplete glycogen stores, while the solution will serve to replace fluid lost as sweat during exercise (Gisolfi and Duchman, 1992). Research has shown that muscle glycogen resynthesis is optimized when carbohydrate is consumed at a rate of approximately 50g every two hours (Coyle, 1995, Gisolfi and Duchman, 1992).

Some research has suggested that the glycemic load (the postprandial glycemic response of a food compared to a reference food (Gretebeck, 2002)), of a pre-exercise carbohydrate meal will affect an individual's performance (Febbraio et al, 2000a). However, the effects of glycemic load (also referred to as the Glycemic Index) have been shown to be negligible to submaximal exercise performance, but high glycemic load (GL) meals do increase carbohydrate utilization during submaximal exercise (Febbraio et al, 2000a). It has been shown that carbohydrate during submaximal exercise minimizes the effect of the GL of the pre-exercise meal on metabolism and performance during exercise (Burke et al, 1998).

2.7 Carbohydrates, Exercise, and The Immune System

Athletes will quite often consume dietary supplements or alter their current diets in attempt to gain an edge in training or competition. This manipulation of diet by an athlete during a period of training may influence innate immunity (Pedersen et al, 2000b). One such substance that is often adjusted by athletes in their diet in attempts to gain an advantage is dietary carbohydrate. Increases in dietary carbohydrate have been shown to

increase the body's glycogen stores (Febbraio et al, 2000b). This can be accomplished by either diet modification or carbohydrate supplementation. The process of carbohydrate supplementation has been found to increase the time to fatigue in endurance athletes (Febbraio et al, 2000b, Lamb and Murray, 1988). Another benefit of increased glycogen stores available to the body during exercise appears to be a reduction in stress hormones, including cortisol levels, during and after exercise (Gleeson and Bishop, 2000). The release of cortisol during and after exercise has been linked to suppression of certain immune cell responses (Nieman, 1997a) as discussed above.

A specific nutrient may impact the immune system directly by triggering immune cell activation or interaction, or indirectly by altering energy metabolism or hormones (Field, 1996). Carbohydrate has been receiving much attention in recent exercise immunology research, as it appears that it may have beneficial effects on immune function. Nieman et al (1998a) have proposed that the maintenance of blood glucose and muscle glycogen levels during exercise through carbohydrate beverage consumption reduces the stimulation of the HPA axis. This results in decreased production of ACTH, which in turn lowers cortisol production. As stated above, cortisol affects certain immune cell responses including the anti-inflammatory response and neutrophil mobilization (Nieman, 1997a). Gleeson and Bishop (2000) have suggested that maintaining adequate amounts of carbohydrate in the diet maintains immunocompetence in an individual by providing sufficient carbohydrate (blood glucose and muscle glycogen) to fuel immune cell metabolism, function, and proliferation.

Gleeson and Bihsop (2000 and 1998), Henson et al (1999), Nieman et al (1998a,b) have found that the consumption of a carbohydrate beverage before, during,

and after exercise has attenuated the expected increases in neutrophil numbers and decrease in leukocyte numbers following endurance exercise. Decreased cortisol levels also accompanied these changes, when compared to a placebo group that received a non-carbohydrate beverage. Bishop et al (2002) found changes in neutrophil trafficking but not activity. Mitchell et al (1998) also found decreases in circulating leukocyte numbers but no differences in lymphocyte proliferation. Bacurau et al (2002) found carbohydrate ingestion prevented the decrease in lymphocyte proliferation seen with placebo ingestion following exercise. Also, Nieman et al (1998b) have found that carbohydrate supplementation decreases the phagocytotic and oxidative burst activity of neutrophils and monocytes following exercise, when compared to a placebo group.

In addition to lower cortisol levels following exercise, carbohydrate supplementation has also been found to attenuate the release of some of the mediating cytokines involved in the inflammatory response. Nehlsen-Cannarella et al (1997) and Starkie et al (2000) have found that the consumption of a carbohydrate beverage before, during, and after endurance exercise attenuates cytokine levels, especially IL-1ra (receptor antagonist) and IL-6, during the recovery from exercise. Bishop et al (2002) found carbohydrate ingestion during exercise reduces plasma IL-6 concentrations but not TNFα. Nieman et al (2001) found carbohydrate to attenuate IL-1ra levels but not IL-6. This response, along with the reduced cortisol levels, shows that carbohydrate feeding reduces both the pro and anti-inflammatory response following muscle damage (Nehlsen-Cannarella et al, 1997). Carbohydrate has also been found to prevent the reduction of (in vivo production) IL-1, IL-2, and TNFα concentrations following exercise, when compared to a placebo group (Bacurau et al, 2002).

Bishop et al (2001c) found no differences in neutrophil numbers or activity, or the hormonal response to exercise between a carbohydrate group and placebo group following cycling to fatigue. The above results demonstrate the somewhat equivocal findings of the effects of carbohydrate on the immune system. It is important to note that a majority of the studies were completed at different intensities and durations. However, while it is generally agreed that the extent of altered immune function post exercise is directly related to the intensity and duration of exercise (Nieman and Pedersen, 1999b), not all of the above results directly support this relationship.

It is important to note that the above results were obtained after prolonged intense exercise. The influence of carbohydrate supplementation on the above parameters during low intensity exercise (Bishop et al, 1999, and Nieman et al, 1999a) and resistance exercise (Koch et al, 2001) has been shown to be minimal. Bishop et al (2002) has recently examined the effect of carbohydrate supplementation on the immune response to high intensity, intermittent exercise. It was found that the supplementation decreased changes in neutrophil trafficking and degranulation, plasma IL-6 concentration, and cortisol (Bishop et al, 2002).

The majority of the research on the influence of carbohydrate supplementation has been limited to supplementation immediately before, during and after exercise. However, some information is available on the effects of carbohydrate loading and high carbohydrate diet on the immune system. Gleeson et al (1998) found higher neutrophil and leukocyte counts, and lower cortisol and glutamine concentrations in subjects that did not complete a 3-day carbohydrate loading period, when compared to the subjects that had carbohydrate loaded. The results from Mitchell et al (1998) were similar after a 2-

day period of high or low carbohydrate diet. This is further supported by Bishop et al (2001a,b) who showed a 3-day high carbohydrate diet influenced neutrophil numbers and the cytokine response (IL-6, IL-10) to cycling to fatigue, when compared to a 3-day low carbohydrate diet. Pedersen et al (2000b) found that NKCA was increased in subjects following 7 weeks of a high carbohydrate diet, when compared to a low carbohydrate diet.

None of the studies that investigated the effects of acute carbohydrate supplementation on post exercise NKCA, showed any difference in lytic activity between carbohydrate and placebo groups.

2.8 The Physiology of Rowing

Rowing exercise involves a very large percentage of the body's muscle mass, as well as the contribution of all three of the body's energy systems. Steinacker et al (1998) found that the body utilizes 70% of whole body muscle mass during rowing. Over the course of a 2000-metre rowing race the anaerobic alactic and lactic, and the aerobic system are all stressed near maximal levels in order to maintain high power outputs of between 450 to 550 W (Steinacker et al, 1998). Metabolic analysis of rowing exercise has suggested that 70 to 80% of the energy contribution comes from the aerobic energy system with the remaining 20 to 30% coming from anaerobic metabolism (Secher, 1993 and Hagerman, 1984). These values have been only shown over distances between 2000 and 2500 metres lasting between 6 to 8.5 minutes. There is no available data on the contributions of each energy system during long endurance rowing, however it is likely that the contribution of the aerobic energy system would account for an even greater percentage of total energy due to lower intensity and longer duration than short, high-

intensity rowing. Due to the large contribution of the aerobic energy system, maximal oxygen consumption (VO_2max) is a major determinant to rowing performance (Kramer et al, 1994). Also, since anaerobic metabolism accounts for a substantial portion of total metabolism, rowers will produce high lactate levels (as high as 25 mmol/l) during exercise (Shephard, 1998). To combat this, rowers have been found to have high buffering capacities (Secher, 1993).

Intense rowing is also known to cause muscle damage, which can be linked to higher levels of stress hormones (Nieman et al, 1999a). Resting cortisol levels (Snegovskaya and Viru, 1993a,b) have been found to be higher and the testosterone to cortisol ratio (an indicator of catabolic/anabolic state) has been found to be lower (Secher, 1993) in rowers.

2.9 The Effect of Rowing on the Immune System

As in other activities, extended periods of prolonged rowing training may cause alterations in the immune response to exercise, and leave the rowers open to infection (Shephard, 1998). However, the incidence of illness in rowers has been reported to be no greater than that of the general population (Budgett and Fuller, 1989). Other research has shown that URTIs are more prevalent in rowing than in other athletic groups including marathon/ultra-marathon and middle-distance runners (Castell et al, 1996).

While the immune response to prolonged, low intensity rowing and short duration, high intensity rowing have been reported, there is no available data on the effects of moderate intensity, prolonged rowing. Nieman's work with the US women's Olympic Rowing Team is the primary source of data on the immune response to prolonged, low intensity exercise. The collection of publications (Nieman et al, 2000a,

1999a, Nehlsen-Cannarella et al, 2000, and Henson et al 2000) that resulted from this work has revealed much information regarding the immune system and rowing. This research found moderate increases in total leukocytes, neutrophils, cortisol levels, and cytokines (IL-1ra, IL-6, IL-8, TNFα) and decreases in total lymphocytes, NK cell numbers following exercise at 57% of VO_2max for 2 hours (with frequent breaks). While these findings definitely show a response by the immune system to rowing, the magnitude of the changes seen in the immune response are not as great as those seen in other types of exercise of similar duration, probably due to the intensity of the exercise. This research also revealed that immune function was no different in elite female rowers when compared to non-athletes, except that resting NKCA was significantly higher in the rowers (Nieman, 1999a).

The acute effects of short duration, high intensity rowing has also been studied following an all-out 2000-m row test (Penkman, 2000). The results revealed increases in total leukocytes, neutrophils, as well as lymphocyte proliferation (in response to mitogen stimulation) and neutrophil activity. These effects were again modest when compared to the responses seen in other exercise bouts but in this case, it is probably due to the duration of the exercise. Nielsen et al (1996) also found similar increases in both changes in immune cell counts and function following a 6-minute maximal row.

As stated above, the large muscle mass required and the high intensities of rowing exercise, can cause muscle damage in short duration, high intensity exercise (Secher, 1993). Muscle damage is known to increase the release of stress hormones (especially cortisol) (Nieman, 1999a), which again are known to suppress certain immune functions (Gleeson and Bishop, 2000) as discussed above.

2.10 Conclusion and Hypothesis

The nature of rowing exercise serves as an excellent mode of exercise to study the acute immune response to exercise. The full-body intense nature of rowing exercise in combination with the high levels of stress hormones experienced by rowers following exercise provides sufficient stress to cause alteration to post exercise immune function. This provides an ideal opportunity to study the potential effects of a nutritional intervention on immune function following exercise.

Furthermore, the metabolic demands of immune cells suggest a possible benefit of ensuring adequate carbohydrate ingestion by the athlete before, during, and after exercise to prevent any suppression of the immune response to rowing exercise. A 1-hour bout of rowing exercise should be of sufficient duration and intensity to significantly lower blood glucose concentration and a portion of liver glycogen stores in the body.

While the effects of high intensity, short duration and long duration, low intensity rowing on the immune system has been studied, the effects of prolonged, moderate intensity rowing exercise on immune function have not been examined.

Based on the above review of the current body of knowledge, it is hypothesized that 1 hour of rowing exercise will cause sufficient stress to alter immune function immediately following and during recovery from exercise, when compared to resting values. Furthermore, it can be hypothesized that carbohydrate supplementation prior to, during, and following a 1-hour row will attenuate the transient alterations in immune function following exercise.

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CHAPTER 3

THE EFFECTS OF 1 HOUR OF ROWING EXERCISE AND CARBOHYDRATE SUPPLEMENTATION ON THE IMMUNE SYSTEM.

3.1 Introduction

Rowing exercise is a highly demanding activity involving a large percentage of the body's muscle mass (Steinacker et al, 1998) and requires a contribution from all three of the body's energy system: the ATP-PC, anaerobic glycolysis, and aerobic energy systems (Secher, 1993). Furthermore, intense exercise is known to stimulate the Hypothalamus-Pituitary-Adrenal (HPA) axis (Nieman and Pedersen, 2002). Stimulation of the HPA axis is known to cause the release of adrenocorticotrophic hormone (ACTH), which causes a subsequent release of cortisol (Gleeson and Bishop, 2000). High levels of stress hormones have been shown to suppress certain immune parameters (Gleeson and Bishop, 2000, Suzuki et al, 1999). These include the synthesis of cytokines, and function of B, T, and NK cells (Weicker and Werle, 1991). Thus the full-body, intense nature of rowing exercise may compromise the immune system of rowers (Shephard, 1998).

Individuals who complete intense exercise bouts or heavy training programs, may leave themselves at risk of infection (Nieman, 2000c). The transient period of suppressed immune function following exercise, has been termed the "Open Window" Theory, which may allow the opportunity for an infection to take hold in the body, especially upper respiratory tract infections (Nieman, 1994).

Some research has suggested that carbohydrate supplementation may have the added benefit of preventing alterations in post exercise immune function, and therefore reduce the risk of infection following intense, prolonged exercise (Nieman and Pedersen,

2002). One of the proposed mechanisms of this beneficial effect of carbohydrate supplementation on post exercise immune function is a reduction of stress hormones due to reduced stimulation of the HPA axis (Nieman and Pedersen, 2002). Other researchers (Gleeson and Bishop, 2000) have suggested the importance of maintaining adequate carbohydrate stores (blood glucose and muscle glycogen) in order to fuel the high rates of metabolism in immune cells (Gleeson and Bishop, 2000).

The purpose of this study was to determine the effect of 1 hour of rowing exercise on post exercise immune function, and if carbohydrate supplementation could alter this post exercise immune response. It is hypothesized that 1 hour of rowing exercise will cause sufficient stress to alter immune function following exercise, when compared to resting values. Furthermore, carbohydrate supplementation prior to, during, and following a 1-hour row will attenuate the transient alterations in immune function following exercise.

3.2 Methods and Procedures

Subjects and Experimental Design

Subjects

Twenty-two volunteer male subjects, with either indoor or on-water rowing experience were recruited from the University of Alberta Rowing Club, the Edmonton Rowing Club or the University of Alberta Fitness and Lifestyle Centre. All subjects had been actively participating in strength and endurance training, and had completed a 2000-metre simulated rowing race in the month leading up to the study. Furthermore, all subjects were capable of completing a 1-hour endurance row. Subjects signed an informed consent form prior to participation and the Faculty of Physical Education and Recreation Research Ethics Committee had previously approved all procedures.

Experimental Design

The subjects completed a 1-hour row for maximum distance at a self-determined power output, on a Concept IIC (Morrisville, VT) rowing ergometer. Subjects rowed at an intense, but self-selected pace and were asked to stay within ± 5 seconds of their chosen 500m split-time. While the pace was self-selected, the row itself was maximal in nature as the rowers were instructed to select and maintain the maximum pace possible for the entire duration of the row. The flywheel resistance was set to a drag factor of 120 on the rowing ergometer for all subjects. In addition to distance, stroke rate, 500m split-time, and average power output were recorded every five minutes throughout the test. Heart rate was also recorded using telemetry from a heart rate monitor (Polar USA, CT). For the 3-day period leading up to the 1-hour row, the subjects' diets were modified to include 55% of the energy from carbohydrate. The subjects were required to fast the night before (beginning at 2200 h the evening before) the 1-hour row. This allowed for a fasted, rested blood sample to be taken early in the morning (7:00am), prior to the test. Blood samples were also taken 5 and 60 minutes after the completion of the 1-hour row. All blood samples were analyzed for ACTH, cortisol, blood glucose, hematocrit, hemoglobin, plasma cytokine proliferation (IL-2, IFNγ), total leukocytes, natural killer cell activity, lymphocyte phenotyping (immunofluorescence), and the number and percentage of circulating neutrophils, lymphocytes, and monocytes. All subjects were given a pre-row meal consisting of CLIF® bars (Berkeley, CA) in an amount equaling 1 g of carbohydrate/kg of body weight (see Appendix A for remaining content of CLIF ® bars). Also, subjects were given 1 L of either a carbohydrate (1 g of CHO/kg of body weight Polycose (Ross Products Division, Abbot Laboratories, Columbus, OH) + dilute

Crystal Light® drink mix (Kraft Canada, Don Mills, ON) + 10 mEq NaCl + water) or placebo beverage (dilute Crystal Light® drink mix + 10 mEq NaCl + water) for the hour before the row. Appendix A contains all nutritional information of the pre-event meal and experimental beverages. The beverages were consumed at a rate of 250ml every 15 minutes during and for the hour after the 1-hour row. The subjects had been matched on 2000-metre simulated rowing race time and randomly assigned to either the carbohydrate supplementation or placebo group. The 2000-metre trial was performed at least three days prior to the subjects' 1-hour row. Preparation and distribution of the beverages was conducted in a double-blind fashion.

Dietary Intake Records

The subjects were required to complete a 3-day dietary record a month prior to the 1-hour endurance row. This required the subjects to record all food and beverages consumed in an easy to use logbook for a 3-day period. Instructions on how to properly use the logbook were given during an orientation meeting. At this meeting, the subjects were given a 1-day dietary log to fill out and return. This allowed the subjects to practice filling out the records and also allowed the investigators to give feedback to insure accuracy in completing the 3-day dietary record. All dietary records were analyzed using commercially available software (ESHA Food Processor (Version 7.9), Salem, OR) to determine the amount of carbohydrate, protein, and fat in each individual's diet, as well as to determine daily caloric intake. After the 3-day diet records were entered, the subjects' intakes were altered to provide 55% of energy from carbohydrate to ensure that all participants in both the carbohydrate and placebo groups were consuming the same amount of carbohydrate in the 3-day period leading up to the 1-hour row. The records

were then copied and returned to the subjects with instructions to follow the altered dietary record as closely as possible for the three days leading up to their 1-hour row. In addition, subjects were instructed to consume all of the food and beverage on the third day of their dietary record by 2200h the night before their 1-hour row to allow for a fasted, resting blood sample to be taken the next morning. The 3-day diet modification attempted to minimize differences in substrate availability and glycogen stores. Refer to Appendix B for a sample page from the diet record used in this study.

Cold/Upper Respiratory Tract Infection Questionnaires

All subjects were given cold/upper respiratory tract infection (URTI) questionnaires in order to determine if there was a significant difference in the incidence of illness between the carbohydrate and placebo groups following the 1-hour row. See Appendix C for a sample of the questionnaire. The questionnaire was used with only minor modifications from a questionnaire previously used and validated in health research (Pizzichini et al, 1998, Meschievitz et al, 1984). Subjects were fully instructed on how to fill out the questionnaire following their 1-hour endurance row. The subjects were asked to complete a separate questionnaire for each cold or URTI contracted in the 14 days following their 1-hour row.

Performance Measures

The subjects' fitness level was measured using three physical tests. These tests included a combined ventilatory threshold and maximum oxygen consumption (VT/VO_2max) test, a 2000-metre simulated rowing race, and a multiple repetition maximum (mRM) strength test for leg and bench press exercises.

2000-metre Simulated Rowing Race

The protocol for the 2000-metre simulated rowing race was set out as previously described in Gillies and Bell (2000). This test was performed on a Concept IIC rowing machine and involves rowing 2000-metre as fast as possible. The flywheel air resistance was standardized at a drag factor of 120 for lightweight rowers (<72.5 kg) and 130 for heavy weight rowers (>72.5 kg) on the rowing ergometer. Weight classification was set according to International Rowing Federation (FISA) standards and drag factor was set on the Concept IIC rowing ergometer according to Rowing Canada guidelines. The time required to complete the 2000-metre distance was recorded, as well as 500m split-times, heart rate (using telemetry), stroke rate and average power output for every 200m interval.

VT/VO_2max Test

The combined VT/VO_2max test was measured during a progressive, incremental exercise test to volitional exhaustion on a Concept IIC rowing machine. The flywheel air resistance was standardized at a drag factor of 120 for lightweight rowers (<72.5 kg) and 130 for heavy weight rowers (>72.5 kg) on the rowing ergometer. Weight classification was set according to International Rowing Federation (FISA) standards and drag factor was set on the Concept IIC rowing ergometer according to Rowing Canada guidelines. Subjects began rowing at 100 Watts (W), with the power output increasing 50 W every two minutes until a respiratory exchange ratio (RER) equal to or greater than 1.05 had been reached (Gillies and Bell, 2000). At this point, the power output was increased each minute by either 25 or 50 W (depending on the strength of the subject) until volitional exhaustion or the attainment of VO_2max. The criteria of VO_2max were a plateau in

oxygen consumption (indicated by a less than 0.100 L/min increase in oxygen consumption during three consecutive 15 second recordings) with increasing power output (Gillies and Bell, 2000). Additional criteria defining VO_2max included a RER greater than 1.1, and the attainment of age-predicted or known maximum heart rate (HR_{max}) (Haykowsky et al, 1998). Ventilatory threshold (VT) was determined as the lowest point on the V_E/VCO_2 (total minute ventilation/expired carbon dioxide) vs. power output curve (Bhambhani and Singh, 1985). During the test, oxygen consumption was measured using a properly calibrated metabolic cart (Sensormedics Horizon MMC, Yorba Linda, CA). Heart rate (using telemetry), average power output, and stroke rate were recorded every minute. The completion of the VT/VO_2max test allowed for the comparison of the 1-hour row intensity as a percentage of maximal and threshold values, as well as to estimate oxygen consumption and fuel utilization during the 1-hour row.

Multiple Repetition Maximum Strength Testing

The multiple repetition maximum (mRM) testing was used to estimate the strength of the subjects by predicting the subjects' 1 repetition maximum (1RM) for bench press and 45° inclined leg press, as previously described by Syrotuik et al (2001). The test was preceded by a general 5-minute warm-up of aerobic exercise on a cycle ergometer with stretching of the major joints/muscle groups. This was followed by a set of 10 repetitions of the strength exercise that would be described as an "easy" intensity. A second, more difficult set, of the strength exercise was performed after 1 to 2 minutes of rest. Finally a third set of the strength exercise after 3 to 4 minutes of rest was performed to determine how much weight could be lifted and lowered 8 to 10 times. This procedure was followed for both bench press and 45° inclined leg press. Proper

technique was explained, demonstrated, and monitored by the researchers for both exercises. The values obtained from the mRM testing were used to estimate the subjects' 1-RM values using commercially available software (Power 5.1, Lincoln, NB).

All of the above physical tests have been previously completed in the Sport Performance Unit at the University of Alberta (Gillies and Bell, 2000 and Haykowsky et al, 1998).

Blood Collection & Analysis

Collection

Blood samples were taken prior to (fasted & rested), and 5 and 60 minutes following a 1-hour row. The blood collected was analyzed for blood glucose, hematocrit, hemoglobin, hormones (cortisol and testosterone), plasma cytokine proliferation (IL-2 and IFNγ), lymphocyte phenotyping (immunofluorescence), total circulating leukocytes, and the circulating number and percentages of lymphocytes, neutrophils, and monocytes. A registered nurse obtained all blood samples, from an arm vein under sterile conditions. The amount of blood taken from each individual per sample was 10 ml in two separate vacutainers coated with K_3 EDTA (Tripotassium ethylene diamine tetraacetic acid; Becton Dickinson Systems, Franklin Lanes, NJ), which is an anticoagulant. One 2 ml sample was taken to the core laboratory at the University of Alberta Hospital for analysis of hematocrit, hemoglobin, and immune cell differential counts. The remaining 8 ml blood sample was used for all functional immune assays, as well as all cytokine, blood glucose, and hormone measurements. All blood samples were kept on ice until analyzed.

Analysis

All differential cell count measurements, hematocrit, and hemoglobin were completed at the Core Laboratory located in the University of Alberta Hospital. These analyses were completed using a Coulter ® STKS Hematology Flow Cytometer (Beckman Coulter; Fullerton, CA). Samples were measured once, but were split and passed through three separate apertures simultaneously. The three apertures were then averaged to obtain a final value. All white blood cells were sized and counted using the "Coulter Principle" which is based on measurable changes in electrical resistance produced by the nonconductive cells in the blood sample.

All functional immune assays and cytokine measurements were completed in the human nutrition lab located in the Department of Agricultural, Food and Nutritional Science at the University of Alberta. Blood glucose and hormone measurements were completed in the Faculty of Physical Education & Recreation's Biochemistry Lab at the University of Alberta.

Lymphocyte Isolation

In order to isolate the lymphocytes from the sample, the sample is spun at 700 ×g for 10 minutes to remove the plasma. The plasma was aliquoted and stored at –80°C for further analysis. The remaining sample (buffy coat and red blood cell (RBC) pellet) was diluted with 4ml (1:1 vol/vol) of 10g/L bovine serum albumin (BSA; Fraction V; Sigma Chemical Co., St. Louis, MO) in phosphate buffered saline (PBS, pH=7.4) and the cell suspension was gently mixed. At this point, 100µl (× 6 wells) of the diluted sample was removed and placed in a pre-conditioned microtitre plate for immunofluorescence (discussed in greater detail below). Peripheral mononuclear cells were isolated and

purified by using a density gradient of Histopaque 1077 (Sigma Chemical Co., St. Louis, MO) in a 1:1 ratio (4 ml over 4ml). The layered gradients were spun at 400 ×g for 30 minutes at room temperature with no brake. The lymphocyte band (at the gradient interface) was removed and washed by adding 10g/L BSA in PBS to the isolated lymphocytes and centrifuging at 250 ×g for 10 minutes at 4°C. One millilitre complete culture medium (CCM) was added to the cell pellet. The CCM consisted of RPMI 1640 (Gibco Life Technologies, Burlington, ON) supplemented with l-glutamine (300mg/L), 40g/L heat-inactivated fetal bovine serum (FBS; Sigma Chemical Co., St. Louis, MO), *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES) buffer (25mM; Gibco Life Technologies, Burlington, ON), 2-mercaptoethanol (2.5μmol/ml), and 100μg/ml antibiotic/antimycotic saline solution (Gibco Life Technologies, Burlington, ON) containing penicillin G sulfate (10,000U/ml), streptomycin sulfate (10,000μg/ml) and amphotericin B (25μg/ml)). The number of lymphocytes in the sample were counted using a hemocytometer and the samples were diluted to a concentration of 1×10^6 cells/ml for both the cytokine determination and natural killer cell activity assay. Trypan blue exclusion was used to determine cell viability, which was greater than 98% for all groups.

Immunofluorescence

The immunofluorescence procedure allows for the phenotyping of peripheral blood mononuclear cells. This is achieved by labeling cells using monoclonal antibodies (mAb) specific to the clusters of differentiation (CD) expressed on the desired cells.

The first step of this procedure was to condition a 96-well microtitre plate with 4% vol/vol Fetal Calf Serum (FCS) in Phosphate Buffered Saline (PBS) solution and incubate for 30 minutes at room temperature. A 100μl aliquot of diluted whole blood

(with 4ml of 10g/L BSA in PBS as described under *Lymphocyte Isolation*) was added to each well and RBCs were lyzed using warm lysis buffer (155mol/L ammonium chloride, 20 mol/L sodium bicarbonate, 0.5 mol/L EDTA). Cells were treated and washed twice with buffers to remove all excess RBCs.

The lymphocytes were stained with the following anti-human mouse monoclonal antibodies (mAbs; Sigma Chemical Co., St. Louis, MO and BD Pharmigen, Mississauga, ON) labeled with FITC (fluorescein isothiocyanate), PE (phycoerythrin), or B (biotin): CD3-B or FITC (T cells), CD4-FITC (T_H cells), CD8-PE or B (T_C or T_S cells), CD14-PE (monocytes), CD16-PE (NK cells and macrophages), CD20-FITC (B cells), CD25-PE (IL-2 receptor α subunit - activated NK, T, B cells, monocytes), CD56-B (NK cells). The samples were then incubated at 4°C for 30 minutes. After the incubation, streptavidin was added to the biotin (B) labeled mAbs, as biotin itself has no colour, but the streptavidin with biotin-tandem conjugate emits quantum red (QR). These samples were incubated again for 30 minutes at 4°C and then washed with 200µl PBS containing FBS (40g/L). After the wash, the cells were fixed with 200µl of paraformaldehyde (10g/L in PBS) with sodium azide as a preservative. All samples were acquired using the same FacScan™ (Becton Dickinson, Sunnyvale, CA) flow cytometer within two days of preparation. Analysis (10 000 cells per mAb combination) was performed on the gated lymphocyte population, which was set to exclude any remaining red blood cells. The samples were acquired based on the relative fluorescence intensity.

For each sample, six different combinations of mAbs were added to a separate well (Table 3-1). In the first well, only an isolated sample of lymphocytes (no mAbs) was added to determine the total number of cells in the sample. WELL 2 contained CD3-

B, CD4-FITC, and CD8-PE to determine the concentration of T cells ($CD3^+$), as well as the concentration of the different types of T cells. Cells that are $CD3^+$ cell surface antigen are classified as T cells (Goldsby et al, 2000). Subsets of T cells include $CD3^+/CD4^+$, which are primarily classified as $CD4^+$ T cells (T_H cells), and $CD3^+/CD8^+$, which are classified as cytotoxic or suppressor T cells (T_C or T_S cells) (Goldsby et al, 2000). In WELL 3, CD4-FITC, CD8-B, and CD25-PE were added to the sample. CD25 is an antigen expressed on activated T, B, NK cells, and monocytes indicating an IL-2 cytokine receptor α subunit site (Goldsby et al, 2000). The cytokine IL-2 targets and stimulates both $CD8^+$ and $CD4^+$, but each type of stimulated T cell elicits a different immune response (Kuby, 1997). Therefore, it is helpful to determine the number of both types of the T cells in the sample that are expressing the IL-2 receptor antigen ($CD4^+/CD25^+$and $CD8^+/CD25^+$). This well also allowed the total number and percentage of $CD25^+$ (activated) cells. In order to determine the percent of natural killer (NK) cells ($CD3^-/16^+/56^+$) in the isolated lymphocytes, $CD3^+$-FITC, CD16-PE and CD56-B were added to WELL 4. The $CD3^-/16^+/56^+$ was used to identify NK cells. WELL 5 contained only CD14-PE mAb, which is used to identify monocytes ($CD14^+$), an important cell in innate immune function. However, in this investigation samples were analyzed to contain only the gated lymphocyte population, so $CD14^+$ cells were not used to determine peripheral blood monocyte concentrations. CD20-FITC was the only mAb added to WELL 6 and was used to determine the concentration of B cells ($CD20^+$) in the sample.

Cytokines Production by Isolated Peripheral Blood Cells

In order to test for a cytokine response in a lymphocyte sample, a mitogen may be added to the sample to stimulate cytokine production. The mitogen used was

phytothemagglutinin (PHA), which is known to activate T cells, and more specifically, a T_H1 response (Goldsby et al, 2000).

The PHA mitogen (25 μg/ml) was added to an isolated lymphocyte sample in a concentration of 1×10^6 cells/ml, and 2 ml of complete culture medium (CCM; described above). The samples were then incubated for 48 hours at 37°C in a controlled atmosphere (5% CO_2, 95% relative humidity) to allow for cytokine proliferation. After the incubation, the samples were placed in the centrifuge for 10 min at 200 ×g at 4°C. Finally, the supernatant was removed from the sample and transferred to microcentrifuge tubes and placed in a –80°C freezer until completion of cytokine analysis.

The concentrations of IL-2 and IFNγ were determined using commercially available enzyme-linked immunosorbent assays (ELISA) kits (OptEIA from BD Pharmigen, Mississauga, ON). All standards and solutions were prepared, and all procedures were followed according to kit specifications. All samples were completed in duplicate to ensure a coefficient of variation less than 15%. Any samples that did not meet this requirement were repeated, again in duplicate. Samples were diluted using assay diluent (10%FCS in PBS) to ensure that the sample fell within the range of the standard curve. The final reaction of the assay was stopped using H_2SO_4 (2M) and the samples were read on a Spectra Max 190 plate reader (Molecular Devices, Sunnyvale, CA) at 450nm (wavelength specified by ELISA kits).

Natural Killer Cell Activity

A sample containing 2 million NK cell-sensitive K562 (target) cells (American Type Tissue Culture Collection, Manassas, VA) were incubated for 1 hour at 37°C with 9.25 MBq/ml Sodium Chromate labeled ^{51}Cr (Amersham Pharmacia Biotech, Montreal,

PQ). The cells were then washed twice in cold PBS and aliquoted into a V-bottom, 96-well microtitre culture plate in a concentration of 2×10^5 cells/ml.

The NK (effector) cells were prepared from an isolated sample of lymphocytes. The lymphocytes were added to the wells of the microtitre plate to give the following Effector to Target (K562 cells) cell ratios: 50:1, 25:1, 12.5:1, 6.25:1, and 3.125:1. The samples were incubated for 4 hours at 37°C, and then spun at 200 ×g for 10 min. Next, 75µl of the supernatant (without disturbing the pellet) was added to a Lumaplate and left overnight. The plates were then read on a TopCount NXT (Canberra Packard, Mississauga, ON) to determine the release of ^{51}Cr.

Each of the above ratios was completed in triplicate for each individual sample at each ratio. The first well contained only ^{51}Cr (no target cells) to determine spontaneous release of ^{51}Cr. The second well contained a solution of Triton X and the labeled target cells to determine maximum release (Triton X is a detergent which lyzed all target cells resulting in maximum release of ^{51}Cr). The third well contained target cells and effector cells. The percent specific lysis achieved by the effector cells was determined using the amount of ^{51}Cr released from the series of three wells for each sample and the following formula:

% Specific Lysis=Sample CPM-Spontaneous CPM / Maximum CPM–Spontaneous CPM

*CPM = Counts Per Million

Furthermore, natural killer cytotoxic activity (NKCA) was corrected on a per cell basis using the number of NK cells in each sample, as determined by immunofluorescence ($CD16^+/56^+$), to give *lytic units* (Bryant et al, 1992). Each lytic unit

(L.U.) is equivalent to the number of effector cells ($\times 10^{-3}$) required to lyse 30% of the target cells.

Hormones

All hormone measurements were completed using commercially available radioimmunoassay (RIA) kits (Diasorin, Stillwater, MN). The assays were completed using plasma removed from the subjects' blood samples, which had been stored in a –80°C freezer until the day of the hormone assay. The samples were collected in a vacutainer containing K_3 EDTA to prevent the blood the sample from clotting. All standards were prepared and all procedures were followed according to the manufacturer's instructions. All samples were completed in duplicate and measured using a 1470 Wizard Automatic Gamma Counter (Wallac Analytical Systems Division, Turku, Finland) set to read the samples at the specifications outlined in the RIA manual. The average coefficient of variation was less than 15% for both hormone assays (**ACTH** 9.56% and **Cortisol** 13.96%).

Blood Glucose

All blood glucose measurements were completed on a Beckman Glucose Analyzer 2 (Beckman Instruments Inc., Fullerton, CA). Again, the blood was collected in a tube containing K_3 EDTA to prevent clotting, and the plasma was removed from the subjects' blood samples after the blood had been separated in a centrifuge. These samples were stored in a –80°C freezer until the day of the assay. All standard set-up and calibration procedures were followed according to the analyzer's instruction manual. Completing all samples in duplicate and periodic calibrations of the analyzer insured the accuracy of the results.

Statistical Analysis

Standard statistical procedures were used to calculate the means and standard deviations for all blood measurements taken at the three time points (rest, and 5 and 60 minutes post exercise) around the 1-hour row test. A 2×3 (2 groups, each measured at 3 time points) repeated measures analysis of variance (ANOVA) was used to detect statistically significant differences in any of the blood or immune parameters over time (REST, POST 5, and POST 60), and/or between experimental groups (carbohydrate or placebo conditions). Any significant F ratios, as determined by ANOVA, were evaluated with a Neuman-Keuls post-hoc test to contrast mean differences. Standard independent *t* tests were used to determine any significant difference in the any of the subject characteristics and performance measurements between the carbohydrate and placebo groups. The level of significance or alpha value was set a priori at $P \leq 0.05$ level for all analyses. A commercially available statistical package (STATISTICA, Oklahoma City, OK) was used for all statistical calculations.

3.3 Results

All values reported are mean ± SD. All results are reported by group as ***CHO*** *(carbohydrate group) and* ***PLA*** *(placebo group).*

Subject Characteristics

The mean age, height, and weight of the subjects in the CHO group were 30.0 ± 8.1 years and 179.3 ± 9.8 cm, and 77.3 ± 7.5 kg respectively. In the placebo the mean age, height, and weight were 26.6 ± 6.7 years, 180.4 ± 5.9 cm, and 75.8 ± 7.8 kg respectively. None of the above measurements were significantly different between the two groups (Table 3-2).

Performance Measurements

All data collected during the completion of the performance is presented in Table 3-2. There were no significant differences for any measurement recorded during the combined VO_2max/VT test, 2000-metre simulated rowing race, or mRM test between the carbohydrate and placebo groups.

1-Hour Row Performance

The performance and physiological variables recorded during the 1-hour row are displayed in Table 3-3. VO_2max, percent of VO_2max, and RER were estimated by matching the average power output from the 1-hour row to the same power output during the VO_2max test. This estimation of metabolic activity during the 1-hour row was used to calculate the energy expended and fuel utilization during the row (Table 3-4) based on the energy contributions of fat and carbohydrate at a particular value of the non-protein RER (Foss, 1998).

Dietary Analysis

The mean amounts of carbohydrate, fat, and protein, as well as total kcal for both groups for the 3-day period leading up to the 1-hour row are shown in Table 3-5. There were no significant differences for any of the measures between groups.

Cold/URTI Questionnaires

There were no questionnaires returned by any subject in either group for the fourteen-day period following the 1-hour row test, suggesting no effect of carbohydrate supplementation on the incidence of illness following the rowing exercise in this investigation.

Complete Blood Cell Counts and Differentials

Hemoglobin

The hemoglobin concentration was significantly higher at POST 5 in both groups when compared to resting values (**CHO** 161.9 ± 8.3 g/L vs. 159.2 ± 5.0 g/L, **PLA** 158.7 ± 6.4 g/L vs. 156.4 ± 6.1 g/L). The POST 60 hemoglobin concentrations were significantly lower in both groups than POST 5 (**CHO** 158.9 ± 7.5 g/L, **PLA** 151.5 ± 4.9 g/L), but not significantly different from REST. There were no significant differences for hemoglobin concentration between groups at any time point (Figure 3-1).

Hematocrit

Hematocrit (the % of packed RBCs / Total blood volume) followed a similar pattern to hemoglobin concentration, as it was significantly elevated in both groups at POST 5 compared to resting values (**CHO** 0.48 ± 0.02 vs. 0.47 ± 0.01, **PLA** 0.47 ± 0.02 vs. 0.46 ± 0.02), and was significantly lower at POST 60 (**CHO** 0.46 ± 0.02, **PLA** 0.45 ± 0.02) in both groups when compared to POST 5. There were no significant differences between groups for hematocrit measurements at any of the time points (Figure 3-2).

Leukocytes

Total circulating leukocytes were significantly elevated at POST 5 when compared to REST in both groups (**CHO** 6.7 ± 1.1 × 10^9 cells/L vs. 5.3 ± 0.9 × 10^9 cells/L, **PLA** 8.3 ± 2.0 × 10^9 cells/L vs. 5.9 ± 0.9 × 10^9 cells/L). Total leukocytes remained elevated sixty minutes after exercise (**CHO** 7.2 ± 1.9 × 10^9 cells/L, **PLA** 7.6 ± 2.5 × 10^9 cells/L). There were no significant differences between groups for circulating leukocytes at any time point (Figure 3-3).

Neutrophils

The total number of circulating neutrophils was significantly higher in both groups five minutes after exercise when compared to resting values (**CHO** $3.7 \pm 0.9 \times 10^9$ cells/L vs. $2.5 \pm 0.6 \times 10^9$ cells/L, **PLA** $4.6 \pm 1.5 \times 10^9$ cells/L vs. $3.0 \pm 0.9 \times 10^9$ cells/L). Circulating neutrophils were significantly higher than both REST and POST 5 in both groups at POST 60 (**CHO** $5.5 \pm 2.0 \times 10^9$ cells/L, **PLA** $5.9 \pm 2.5 \times 10^9$ cells/L). There were no differences between groups at any time point (Figure 3-4).

The percentage of neutrophils (expressed as a percentage of total circulating leukocytes) followed a similar pattern to the post exercise changes in circulating neutrophils. Neutrophil percentage was significantly higher in both groups at POST 5 when compared to REST (**CHO** 56.5 ± 7.4 % vs. 47.6 ± 10.4 %, **PLA** 55.6 ± 8.1 % vs. 50.5 ± 8.8 %), and continued to rise significantly at POST 60 (**CHO** 74.3 ± 9.3 %, **PLA** 75.0 ± 9.2 %). There was no effect of group on neutrophil percentage at any time point (Figure 3-5).

Lymphocytes

The total number of circulating lymphocytes was significantly higher five minutes after exercise in the placebo group only, when compared to resting values (**PLA** $2.9 \pm 0.6 \times 10^9$ cells/L vs. $2.1 \pm 0.3 \times 10^9$ cells/L). Circulating lymphocytes were significantly lower than REST and POST 5 in both groups at POST 60 (**CHO** $1.1 \pm 0.3 \times 10^9$ cells, **PLA** $1.1 \pm 0.4 \times 10^9$ cells/L vs. $2.1 \pm 0.9 \times 10^9$ cells/L (REST) and $2.1 \pm 0.6 \times 10^9$ cells, (POST 5)) (Figure 3-6).

The percentage of lymphocytes (expressed as a percentage of total circulating leukocytes) was significantly lower in both groups at POST 60 (**CHO** 15.6 ± 6.9 %, **PLA**

16.6 ± 8.6 %) when compared to REST (**CHO** 38.8 ± 11.0 %, **PLA** 36.4 ± 7.5 %) and POST 5 (**CHO** 31.9 ± 7.6 %, **PLA** 34.7 ± 8.6 %). There was no effect of group on lymphocyte percentage at any time point (Figure 3-7).

Monocytes

The total number of circulating monocytes was not significantly different at any time point in either the carbohydrate or placebo group (Figure 3-8).

However, the percentage of monocytes (expressed as a percentage of total circulating leukocytes) was significantly lower in both groups at POST 5 when compared to REST (**CHO** 8.4 ± 2.2 % vs. 9.5 ± 2.5 %, **PLA** 7.0 ± 1.5 % vs. 8.7 ± 2.4 %). At POST 60, monocyte percentage was significantly lower than both REST and POST 5 (**CHO** 7.9 ± 3.0 %, **PLA** 6.5 ± 2.1 %). There were no significant differences in monocyte percentage between the carbohydrate and placebo groups at any time point (Figure 3-9).

Neutrophil:Lymphocyte Ratio

Nieman (1995) has proposed this ratio as an indicator of physiological stress. The neutrophil:lymphocyte ratio (calculated as Circulating Neutrophils ($\times 10^9$ cells/L) / Circulating Lymphocytes ($\times 10^9$ cells/L)) was significantly elevated sixty minutes (**CHO** 5.9 ± 3.2, **PLA** 6.3 ± 4.4) following exercise when compared to values from rest (**CHO** 1.4 ± 0.6, **PLA** 1.5 ± 0.6) and five minutes (**CHO** 1.9 ± 0.8, **PLA** 1.6 ± 0.5) after exercise. There was no effect of group on the neutrophil:lymphocyte ratio at any time point (Figure 3-10).

Cytokine Production

The IL-2 production was significantly lower in both groups from lymphocytes isolated from the POST 5 samples compared to REST (**CHO** 206.0 ± 260.9 pg/ml vs. 499.6 ± 407.0 pg/ml, **PLA** 306.9 ± 294.3 pg/ml vs. 435.6 ± 436.8 pg/ml). IL-2 production remained significantly lower than REST at POST 60 (**CHO** 244.3 ± 221.7, **PLA** 246.2 ± 297.5). There were no differences in IL-2 concentration between groups at any of the time points (Figure 3-11).

The IFNγ concentration was not significantly different at POST 5 when compared to REST values. However, IFNγ production was significantly reduced in both groups at POST 60 (**CHO** 394.5 ± 387.3 pg/ml, **PLA** 336.5 ± 336.3 pg/ml) when compared to both POST 5 (**CHO** 811.1 ± 577.6 pg/ml, **PLA** 807.0 ± 583.8 pg/ml) and REST (**CHO** 881.8 ± 574.8 pg/ml, **PLA** 785.0 ± 471.6 pg/ml). There were no significant differences between groups for IFNγ concentration at any time point (Figure 3-12).

Natural Killer Cell Activity

When expressed at percent specific lysis, NKCA was significantly depressed at POST 60 in both groups when compared to REST and POST 5 for the following effector to target cell ratios: 6.25:1 and 12.5:1. At an effector to target cell ratio of 25:1, NKCA was significantly depressed in both groups at POST 60 when compared to POST 5. There were no significant differences in NKCA for the target to effector cell ratios of 3.125:1 and 50:1, at any of the time points. There was no effect of carbohydrate supplementation on NKCA at any time point or any effector to target ratio (Table 3-6).

When expressed in lytic units (the amount of effector ($CD16^+/56^+$) cells × 10^3 required to kill 30% of target cells), NKCA was significantly higher in both groups

immediately after exercise when compared to resting values (**CHO** 13.81 ± 12.88 L.U. vs. 6.88 ± 5.99 L.U., **PLA** 10.94 ± 8.56 L.U. vs. 7.68 ± 5.49 L.U.). NKCA had returned near REST values at POST 60 (**CHO** 6.43 ± 2.49 L.U., **PLA** 7.55 ± 4.91 L.U.). There were no significant differences between the carbohydrate and placebo groups for NKCA at any time point (Table 3-7).

Immunofluorescence

The proportions of the individual cell types obtained from the immunofluorescence procedure (data is shown in Table 3-8) and the lymphocyte concentration obtained from the complete blood counts (CBCs) was used to calculate the concentration ($\times 10^9$ cells/L) of the different cells in the sample. All cell concentrations are shown in Table 3-9.

In the first well, the concentration of T cells ($CD3^+$), as well as $CD4^+$ and $CD8^+$ T cells in the blood samples was determined. The total number of $CD3^+$ cells remained unchanged in both groups immediately following exercise when compared to resting values (**CHO** $1.36 \pm 0.50 \times 10^9$ cells/L vs. $1.46 \pm 0.82 \times 10^9$ cells/L, **PLA** $1.79 \pm 0.38 \times 10^9$ cells/L vs. $1.41 \pm 0.57 \times 10^9$ cells/L), but decreased significantly after an hour of recovery (**CHO** $0.78 \pm 0.39 \times 10^9$ cells/L, **PLA** $0.79 \pm 0.28 \times 10^9$ cells/L). The concentration of $CD3^+/4^+$ cells followed a similar pattern as it remained unchanged immediately following exercise when compared to resting values (**CHO** $0.78 \pm 0.33 \times 10^9$ cells/L vs. $0.90 \pm 0.65 \times 10^9$ cells/L, **PLA** $0.92 \pm 0.31 \times 10^9$ cells/L vs. $0.94 \pm 0.42 \times 10^9$ cells/L), but again decreased similarly in both groups after an hour of recovery (**CHO** $0.45 \pm 0.29 \times 10^9$ cells/L, **PLA** $0.53 \pm 0.23 \times 10^9$ cells/L). This pattern was again seen in the concentration of $CD3^+/8^+$ cells. The POST 5 concentration in both groups (**CHO**

0.60 ± 0.38 × 10^9 cells/L, **PLA** 0.87 ± 0.46 × 10^9 cells/L) was not significantly different than the resting values (**CHO** 0.58 ± 0.42 × 10^9 cells/L, **PLA** 0.65 ± 0.31 × 10^9 cells/L), but $CD3^+/8^+$ concentrations were significantly lower 60 minutes after exercise (**CHO** 0.33 ± 0.35 × 10^9 cells/L, **PLA** 0.41 ± 0.20 × 10^9 cells/L). There were no significant differences for any of the above cell concentrations at any of the time points between the carbohydrate and placebo groups.

In the second well, the concentration of activated $CD4^+$ and $CD8^+$ T cells, as well as the total concentration of CD25+ cells was determined. The total concentration of $CD25^+$ cells remained unchanged immediately following exercise when compared to resting values in both groups (**CHO** 0.19 ± 0.10 × 10^9 cells/L vs. 0.22 ± 0.15 × 10^9 cells/L, **PLA** 0.34 ± 0.21 × 10^9 cells/L vs. 0.28 ± 0.13 × 10^9 cells/L), but decreased significantly after an hour of recovery (**CHO** 0.19 ± 0.21 × 10^9 cells/L, **PLA** 0.14 ± 0.08 × 10^9 cells/L). The concentration of $CD4^+/25^+$ cells followed a similar pattern in both groups as it remained unchanged immediately following exercise when compared to resting values (**CHO** 0.12 ± 0.06 × 10^9 cells/L vs. 0.11 ± 0.09 × 10^9 cells/L, **PLA** 0.19 ± 0.13 × 10^9 cells/L vs. 0.18 ± 0.09 × 10^9 cells/L), but again decreased significantly after an hour of recovery (**CHO** 0.09 ± 0.09 × 10^9 cells/L, **PLA** 0.10 ± 0.06 × 10^9 cells/L). The concentration of $CD8^+/25^+$ was not significantly different at any of the three time points. There were no significant differences for any of the above cell concentrations at any of the time points between the carbohydrate and placebo groups.

In the third well, the concentrations of $CD3^-/16^+$, CD3-/56+, and CD3-/16+/56+ cells were determined. The concentration of $CD3^-/16^+$ was significantly elevated in the placebo group only five minutes after exercise compared to rest (**PLA** 0.67 ± 0.10 × 10^9

cells/L vs. 0.30 ± 0.22 × 10^9 cells/L) where as the concentration remained unchanged in the carbohydrate group (**CHO** 0.32 ± 0.38 × 10^9 cells/L vs. 0.21 ± 0.20 × 10^9 cells/L). The concentration of $CD3^-/16^+$ cells was significantly lower in both groups an hour after exercise (**CHO** 0.08 ± 0.70 × 10^9 cells/L, **PLA** 0.12 ± 0.08 × 10^9 cells/L). The concentration of $CD3^-/56^+$ cells was significantly elevated in both groups five minutes after exercise (**CHO** 0.23 ± 0.19 × 10^9 cells/L vs. 0.14 ± 0.17 × 10^9 cells/L, **PLA** 0.51 ± 0.40 × 10^9 cells/L vs. 0.19 ± 0.19 × 10^9 cells/L), and returned to near resting values by POST 60 (**CHO** 0.08 ± 0.08 × 10^9 cells/L, **PLA** 0.10 ± 0.09 × 10^9 cells/L). The concentration of $CD3^-/16^+/56^+$ cells followed a similar pattern, as it was significantly elevated in both groups at POST 5 (**CHO** 0.29 ± 0.19 × 10^9 cells/L vs. 0.18 ± 0.14 × 10^9 cells/L, **PLA** 0.51 ± 0.40 × 10^9 cells/L vs. 0.18 ± 0.17 × 10^9 cells/L), and had returned to near resting values by POST 60 (**CHO** 0.08 ± 0.06 × 10^9 cells/L, **PLA** 0.07 ± 0.04 × 10^9 cells/L).

Well five was used to determine the concentration of $CD20^+$ cells in the samples. The $CD20^+$ concentration was significantly elevated in both groups immediately after exercise (**CHO** 0.19 ± 0.07 × 10^9 cells/L vs. 0.21 ± 0.15 × 10^9 cells/L, **PLA** 0.26 ± 0.08 × 10^9 cells/L vs. 0.15 ± 0.08 × 10^9 cells/L), and was significantly lower after an hour of recovery from exercise (**CHO** 0.11 ± 0.05 × 10^9 cells/L, **PLA** 0.10 ± 0.06 × 10^9 cells/L). There was no significant difference between the carbohydrate and placebo groups at any time point for $CD20^+$ concentrations.

CD4:CD8 Ratio

Much like the neutrophil to lymphocyte ratio, the CD4 to CD8 ratio can be used as an indicator of physiological stress and is often tracked in the literature during and

after exercise (Mackinnon, 1999). The ratio of $CD4^+$ to $CD8^+$ T cells is approximately 2:1 at rest in human peripheral blood (Goldsby et al, 2000) and has been found to decrease during and after exercise (Mackinnon, 1999). This ratio was determined by simply dividing the number of $CD3^+/4^+$ cells by the number of $CD3^+/8^+$ cells and is displayed on Table 3-10. In this investigation, the CD4 to CD8 ratio remained unchanged from resting values (**CHO** 1.7 ± 0.9, **PLA** 1.6 ± 0.9) at both the POST 5 (**CHO** 1.6 ± 0.8, **PLA** 1.3 ± 0.7) and POST 60 (**CHO** 2.1 ± 2.1, **PLA** 1.7 ± 1.1) time points. There was also no significant difference between the carbohydrate and placebo groups for the CD4 to CD8 ratio at any time point.

Hormones

ACTH levels were significantly elevated in both groups five minutes following exercise (**CHO** 91.7 ± 53.8 pg/ml vs. 35.6 ± 13.3 pg/ml, **PLA** 115.8 ± 95.7 pg/ml vs. 31.9 ± 13.2 pg/ml). ACTH levels had returned to near resting levels an hour after exercise (Figure 3-13).

Much like ACTH, cortisol levels were significantly elevated in both groups at POST 5 when compared to REST (**CHO** 31.3 ± 8.6 μg/dl vs. 20.2 ± 4.4 μg/dl, **PLA** 31.5 ± 11.4 μg/dl vs. 19.2 ± 2.4 μg/dl). By POST 60, the cortisol levels had returned to near resting values (see Figure 3-14).

There were no significant differences for ACTH or cortisol measurements at any time point between the carbohydrate and placebo groups.

Blood Glucose

Blood glucose concentration was significantly elevated in both groups at POST 5 when compared to REST (**CHO** 7.30 ± 1.54 mmol/L vs. 4.53 ± 0.36 mmol/L, **PLA** 5.50

± 0.84 mmol/L vs. 4.69 ± 0.53 mmol/L). These elevated levels had returned to near resting values by POST 60 (Figure 3-15). Blood glucose concentration was significantly higher in the CHO group at POST 5 when compared to the PLA group (7.30 ± 1.54 mmol/L vs. 5.50 ± 0.84 mmol/L).

3.4 Discussion

This investigation had two main purposes: to examine the effect of 1 hour of rowing exercise on post exercise immune function and to determine the effect of carbohydrate supplementation on any changes in immune function following exercise. It was hypothesized that 1 hour of rowing exercise would alter immune cell counts and function post exercise. All of the measurements that were completed to indicate immune function were significantly altered following exercise (either at POST 5, POST 60, or both) when compared to resting values, except for circulating monocyte numbers (determined by differential white blood cell counts), and $CD8^+$ T cells ($CD8^+/CD25^+$) expressing the IL-2 α receptor subunit ($CD25^+$) (determined by immunofluorescence). The changes in post exercise immune cell counts and function, for the most part, paralleled exercise-induced changes in circulating levels of stress hormones (ACTH and cortisol). Furthermore, it was hypothesized that carbohydrate would attenuate these changes following exercise through the maintenance of blood glucose and a reduced production of stress hormones. The only immune alterations that were attenuated following exercise by carbohydrate supplementation were the total number of circulating lymphocytes (determined by differential immune cell counts) and the concentration of $CD3^-/16^+$ cells (monocytes and NK cells; determined by immunofluorescence), both at five minutes after exercise. It is important to note that none of the individual types of the

circulating lymphocytes ($CD4^+$, $CD8^+$, B, and NK cells) were significantly different between the carbohydrate and placebo groups at the POST 5 measurement despite the observed significant difference in total circulating lymphocytes. Therefore the physiological relevance of the observed effect of carbohydrate supplementation on lymphocyte concentrations is questionable.

The measures obtained for immune cell counts and function after exercise indicated that immune system was significantly altered following 1 hour of rowing exercise, when compared to resting values. The results were also consistent with patterns of change observed in post exercise immune function after other bouts of long duration, intense exercise (Mackinnon, 1999). At this point, it is important to note that the designation "long duration, intense" exercise fits only when comparing the duration and intensity of exercise in this investigation to the exercise immunology literature (Mackinnon, 1999, Shephard and Shek, 1999). However, when compared to the general exercise literature, the intensity of exercise in this investigation would be classified as "moderate".

The post exercise immune cell concentrations from this investigation will now be briefly reviewed. The total concentration of circulating leukocytes (WBCs) in the blood immediately following exercise was significantly elevated (33%) and remained significantly elevated (24%) compared to rest an hour after exercise. This response is commonly referred to as *leukocytosis*. The concentration of neutrophils was also significantly elevated immediately following exercise (52%) and continued to rise after an hour of recovery (107%). This rise in neutrophil concentration is often referred to as neutrophilia. The circulating levels of total lymphocytes in the blood five minutes after

exercise were significantly elevated (20%, in the placebo group only) and fell significantly below resting values (49%) at the POST 60 measurement (in both groups). This is often referred to as a biphasic response of lymphocytes in the literature. The post exercise response of the individual lymphocytes will now be briefly presented. The concentration of NK ($CD3^-/16^+/56^+$) and B cells ($CD20^+$) were significantly elevated (122% and 25% respectively), while the total T cells ($CD3^+$) as well as $CD4^+$ and $CD8^+$ T cells remained unchanged from resting values immediately following exercise. After an hour of recovery, the concentration of NK cells had returned to near resting values, while total T (45%), $CD4^+$ (47%), $CD8^+$ (40%), and B cells (42%) were all significantly lower than resting values. There was no effect of exercise on the concentration of monocytes in peripheral blood.

While a majority of the post exercise immune responses were consistent with those patterns described in the current body of research (Mackinnon, 1999), a few of the response in this investigation were not consistent with the post exercise changes reported in the literature. As stated above, the circulating monocyte numbers did not significantly change following exercise. This is inconsistent with the literature, which has generally found monocyte levels to increase immediately following exercise and remain elevated after one hour of recovery (Mackinnon, 1999). One possible explanation for this finding may be that monocytes are known to localize to sites of muscle damage, where they differentiate into macrophages (Mackinnon, 1999), which could possibly be increased to the muscle damage often seen following rowing exercise (Secher, 1993). Also, B cells ($CD20^+$) percentage remained unchanged immediately post exercise, but was significantly elevated an hour after exercise. A review of the exercise immunology

research suggests no change in B cell ($CD20^+$) either immediately following exercise or during recovery from exercise (Mackinnon, 1999) or only slight changes in B cell proportions, which do not persist long after exercise (Neiman and Pedersen, 2002, Nielsen, 1996). Therefore, the significant increase in B cells seen in this investigation immediately following exercise and the subsequent decrease after an hour of recovery is not supported by the literature. This significant post exercise change of B cells cannot be explained and may not be significant to the overall functioning of the immune system immediately following exercise, as the increase was not great. Finally, as stated above, the concentrations of T cells, $CD4^+$, and $CD8^+$ T cells did not increase significantly from resting values immediately after exercise. This contrasts the literature, which suggest an increase would be seen in all of these measures immediately after exercise (Mackinnon, 1999).

Nieman et al (1995) have suggested that looking at the neutrophil to lymphocyte ratio may be a useful way of evaluating physiological stress following exercise. The level of physiological stress is related to the extent of change in immune function following exercise. As previously stated, neutrophils account for the majority of circulating leukocytes both at rest and following exercise, and lymphocytes experience an initial increase immediately post exercise and then drop below resting levels during recovery (Mackinnon, 1999). Therefore, the neutrophil to lymphocyte ratio should increase immediately following exercise and then continue to increase during recovery as neutrophils continue to rise and lymphocyte numbers drop. The extent to which the ratio rises during recovery is therefore directly related to the physiological stress caused by the interaction of both the duration and intensity of the exercise (Nieman et al, 1995). The

change in the neutrophil to lymphocyte ratio measured in this investigation is not congruent with the above investigation immediately after exercise, as there was no significant increase in the ratio. This may be due to lower absolute increases in both circulating neutrophils and lymphocytes when compared to other research. However, the neutrophil to lymphocyte ratio was elevated significantly compared to rest after sixty minutes of recovery, as is reported in the literature (Nieman et al, 1995). Another measure that can be used to determine physiological stress is the $CD4^+$ to $CD8^+$ ratio. This ratio has been found to decrease immediately following exercise, as $CD8^+$ cells increase disproportionately to $CD4^+$ cells causing the ratio to decrease (Mackinnon, 1999). However, in this investigation the $CD4^+$ to $CD8^+$ ratio remained unchanged from resting levels, both five and sixty minutes after the rowing exercise. This can be explained by the fact that the concentrations of both of these cells remained unchanged immediately following exercise, and decreased proportionately (47% for $CD4^+$ and 40% for $CD8^+$) after an hour of recovery.

The above responses clearly indicate that one hour of rowing exercise is sufficient to change most circulating leukocytes concentrations. The primary cause of the exercise-induced leukocytosis is an increase in neutrophils or neutrophillia (Mackinnon, 1999). This is consistent with the results of this study, as neutrophils accounted for over 50 % of the total circulating leukocytes immediately following exercise, and more than 70% an hour after the row. The primary mechanism suggested for this increased release of neutrophils from the bone marrow during and following exercise is the stimulation of the Hypothalamus-Pituitary- Adrenal (HPA) axis, and the subsequent release of stress

hormones (adrenocorticotrophic hormone (ACTH) and cortisol) (Gleeson and Bishop, 2000, Utter et al, 1999, Mackinnon, 1999).

This mechanism is supported by the results of this investigation as the concentrations of both ACTH and cortisol were significantly elevated, 208% and 59% greater than rest respectively, five minutes after exercise, and returned to near resting levels sixty minutes after exercise. The research of Nieman et al (1999), Henson et al (1999), and Utter et al (1999) supports this cortisol response following exercise. The ACTH response to exercise has not been extensively studied in the exercise immunology literature despite being suggested as a mechanism of post exercise immune alterations. Duclos et al (1997) reported a significant increase in ACTH concentration following 120 minutes of exercise at 80% of VO_2max. Ronsen et al (2001) found significant increases in ACTH levels following exercise at 75% of VO_2max for 65 minutes. In addition, it was also found that ACTH and cortisol levels dropped significantly with no activity (75% and 80% decreases respectively) between 7:30am and 8:30am due to diurnal changes in the hormones levels (Ronsen et al, 2001). Since the REST hormone measurements for this investigation were taken at 7:00am, the actual value immediately before exercise may have been even lower than those taken at rest. All blood samples at the three time points were taken from the subjects as close as possible to the same time of day (except for one, with REST sample obtained at that the same time (7:00am), but POST 5 and 60 were taken approximately one hour later than the other subjects). Therefore, the effects of diurnal rhythm on hormone concentrations in the blood samples between subjects would have been minimal.

The functional immune cell assay chosen for this study was to measure natural killer cell cytotoxic activity (NKCA), due the importance of NK cells in the early stages of infection (Kuby, 1997). The findings of this study in regards to NKCA (expressed as percent specific lysis) are congruent with the literature (Mackinnon, 1999), as it was significantly lower than resting values during recovery from exercise (at Target to Effector Ratios of 6.25:1 and 12.5:1). However, there was no significant increase immediately following exercise that has been reported previously by Henson et al (1999) and Nieman (1997). When corrected on a per cell basis to lytic units, NKCA did increase immediately after exercise, however, the activity returned to resting levels following sixty minutes of recovery, but did not significantly drop below resting values. The findings of Henson et al (1999) and Shephard and Shek (1999) support the pattern of change seen in lytic units. One of the suggested mechanisms of the pattern of response of lytic activity to exercise is simply that it increases or decreases according to the circulating NK cells in the blood (Shephard and Shek, 1999). This is supported by our investigation as changes in NKCA paralleled the exercise-induced changes in circulating NK cells ($CD16^{+}/56^{+}$). Another proposed mechanism of post exercise alterations in NKCA is that the activation level or sensitivity to other stimulatory factors is increased in NK cells by exercise (Mackinnon, 1999). This includes sensitivity to certain cytokines, such as IL-2, which is known to stimulate NKCA (Goldsby et al, 2000).

Cytokine production was measured in vitro by stimulating the release of IL-2 and IFNγ from an isolated lymphocyte sample using phytothemagglutinin (PHA) mitogen. PHA is known to stimulate a T_H1 or primarily cytotoxic response, which activates the release of a number of cytokines (Goldsby et al, 2000), including the two of interest to

this investigation. The production of IL-2 decreased immediately following exercise (45%), and remained significantly lower (48%) than resting values after sixty minutes of exercise. This occurred when there was no change in the relative percentage of CD3+, CD4+ or CD8+ cells in the assay. This is supported in the literature, which reports a decrease in IL-2 production immediately following exercise (Mackinnon, 1999) and one hour after exercise (Shephard et al, 1994). Although NK assay was not measured under these same conditions, the lower ability of cells to produce IL-2 may be of physiological importance to NK activity. IL-2 is known to stimulate NKCA (Goldsby et al, 2000), thus a decreased production of IL-2 could cause a subsequent decrease in NKCA.

IFNγ production remained unchanged five minutes post exercise, but was significantly depressed (58%) at sixty minutes after exercise. This is supported by Northoff et al (1998) who found similar decreases in the production of IFNγ in vivo one hour after exercise. Shephard et al (1994) has suggested that IL-2 may cause the release of other cytokines, including IFNγ. However, the production of these two cytokines in the current investigation appear to be unrelated as mitogen-induced IL-2 concentration decreased immediately after exercise and remained depressed throughout exercise, where as mitogen-stimulated IFNγ remained unchanged from rest immediately after exercise and was significantly depressed an hour after exercise. The suppression of mitogen-stimulated IFNγ after an exercise bout has been suggested as one of the main reasons of immune suppression following strenuous exercise and the possible increase in infection risk during this period of transient immune function (Northoff et al, 1998). Northoff et al (1998) have also suggested that the decrease ability to produce IFNγ following exercise is responsible for the post exercise alterations in leukocytes, and suppressed NKCA and B

cell activity. Northoff et al (1998) has also suggested that the suppression of IFNγ may be an attempt to counter balance the inflammatory response due to muscle damage, which is often seen following rowing exercise (Nieman, 1999).

In summary, a large majority of the above responses of immune cell concentrations, function, and in vivo cytokine production clearly indicate an altered post exercise immune function following 1 hour of rowing exercise. While many of the changes had returned to near resting values an hour after the rowing exercise, it is unclear how long the alterations lasted or what the pattern of changes were in immune function beyond the hour of recovery following the rowing exercise bout.

Despite the post exercise changes being similar to those found in the current body of research, many of the changes following exercise in the literature are greater than those seen in this investigation. Due to the relationship between exercise duration and intensity, and the extent of post exercise immune response (Nieman, 2000) the observed differences in this investigation may be due to the intensity (≈72% of VO_2max and ≈93% of VT) and duration (1 hour) of the rowing exercise in this investigation. It is not surprising that the post exercise alterations of immune function were not as exaggerated in this study when compared to exercise bouts of longer duration (Henson et al, 1999 (2.5 hrs @ 75% of VO_2max and 100% of VT), Nieman et al, 1997 (2.5 hrs @ 75% of VO_2max and 100% of VT)) and higher intensity (Bishop et al, 2002 (Maximal exercise for 15 min × 6)) or both (Bishop et al, 2001a,b (1 hrs @ 60% VO_2max + 0.5 hrs @ 80% of VO_2max). Furthermore, the influence of carbohydrate on stress hormone concentrations, immune cell distribution and some functional capacities in these studies was greater as well.

When comparing the findings of this investigation to an exercise bout of lower intensity (Henson et al, 2000, Nieman et al, 1999) some interesting comparisons can be made. While the subjects in these studies rowed for a longer period than the subjects in our study (2 hours with 18 minutes rest vs. 1 hour), they rowed at a much lower intensity (57% VO_2max vs. 72% of VO_2max) with frequent breaks. Despite the considerably lower intensity, carbohydrate attenuated the rise of neutrophils, monocytes, total leukocytes, and phagocytotic activity following exercise, but did not alter stress hormone concentration or lymphocyte subsets. The above responses that were influenced by carbohydrate supplementation, which are greater than those seen in our investigation must then be due to the duration of the exercise. This is supported by Mackinnon (1999), who suggests that the duration of exercise has more effect on the post exercise immune response than the intensity. However, Starkie et al (2000) found no effect of carbohydrate on cytokine production or circulating leukocyte numbers following 2 hours of cycling at 70% VO_2max. The conflicting results of these studies are not in agreement with the generally accepted relationship between intensity, duration, and extent of the post exercise immune response (Nieman and Pedersen, 2002). However, they have demonstrated the importance of carefully selecting a bout of exercise of sufficient intensity and duration to sufficiently alter the immune response and deplete glycogen stores in order to maximize the effect of carbohydrate supplementation. Furthermore, the equivocal findings of these studies may be accounted for by the subjects in Nieman's (1999a) investigation (female Olympic-level rowers). Females have been shown to have a hypercortisol response to exercise (Bell et al, 1997), which may have increased the extent of post exercise immune changes and furthermore the effect of carbohydrate

supplementation on the immune alterations. In addition, the subjects may already have been experiencing altered immune function due to the additive effects of repeated training bouts or possibly even overtraining (Mackinnon, 1999).

When comparing exercise bouts of both similar duration and intensity to our study, the effects of carbohydrate supplementation are unclear. Bishop et al (2001c), found no effect of carbohydrate on total leukocytes, lymphocytes, neutrophil counts or activity, or circulating hormones (cortisol or insulin) after cycling exercise at 75% of VO_2max until fatigue (≈ 77 to 98 min). While these findings are in agreement with our investigation, other studies have found conflicting results. Bacurau et al (2002), found significant effects of carbohydrate supplementation on lymphocyte proliferation, cortisol concentration, and in vivo cytokine production (IL-1, IL-2, TNFα) following 6 sets of (20 minutes on, 20 minutes off) cycling at 90% of anaerobic threshold. Mitchell et al (1998) found carbohydrate to attenuate the increases in leukocytes and cortisol following an hour of cycling at 75% of VO_2max. Gleeson et al (1998) also found a decrease in the post exercise leukocytosis and a reduction of cortisol in a carbohydrate group compared to a placebo group following 1 hour of cycling at 70% of VO_2max. It is important to note that the last studies mentioned use a period of carbohydrate loading (2 and 3 days respectively) as opposed to acute carbohydrate supplementation. This may suggest adequate carbohydrate in the diet is more important to post exercise immune function than supplementation with carbohydrate immediately before, during, and after exercise. One possible explanation of the data set from our investigation, which conflicts with the previous three studies mentioned, is the inclusion of a pre-event meal in our experimental design. All subjects were given a meal consisting of 1 g of CHO/kg body weight, in

addition to their placebo or carbohydrate beverage. This meal followed pre-event nutrition guidelines (amount of CHO, timing) as previously outlined by Sherman (1989) to optimize carbohydrate availability for endurance performance. This pre-event meal may have maintained blood glucose concentrations enough in the placebo group (despite still being significantly lower than the carbohydrate group immediately following exercise) to reduce the stimulation of the HPA axis and the subsequent release of ACTH and cortisol. Therefore, in actuality, carbohydrate may have attenuated the immune responses of the subjects in both groups to a certain extent, which could somewhat explain the significant effect of carbohydrate on only two immune measures. This is supported by Sherman et al (1991) who reported that despite differences in blood glucose and insulin responses, both a 1.1 g and 2.2g of CHO/kg of body weight pre-exercise meal (1 hour before) improved performance in cycling at 70% of VO_2max. This may indicate that subjects in both groups were able to exercise at a similar intensity for the 1-hour row, regardless of the beverage consumed, and therefore had similar hormonal and immune responses.

Despite blood glucose levels being significantly elevated in the carbohydrate group five minutes after exercise, the only immune changes that were attenuated were circulating lymphocyte number and $CD3^-/CD16^+$ (monocytes and NK cells) concentration as determined by immunofluorescence. The attenuation of the post exercise rise in these variables is consistent with other studies that have measured immune parameters following exercise with carbohydrate supplementation (Henson et al, 1999, Gleeson et al, 1998). However, these studies saw attenuation of all leukocytes when compared to placebo ingestion. It is also important to note that carbohydrate

supplementation did not appear to give any advantage in protecting from infection, as no illness were reported by subjects in either group in the 14 days following the 1-hour row. Furthermore, when stress hormones were measured following exercise, carbohydrate supplementation has been correlated to lower concentrations of cortisol (Bacurau et al, 2002, Henson et al, 1999, Utter et al, 1999). Nieman (1999a) has suggested that this is achieved by reduced stimulation of the HPA axis by the maintenance of blood glucose concentrations. However, in this investigation, both ACTH and cortisol increased similarly in both groups immediately following exercise, despite a higher blood glucose concentration in the carbohydrate group at the same time point. One possible explanation of this equivocal finding is that the subjects in the others studies were not given a pre-event meal. In this investigation, all subjects were given a pre-event meal consisting of 1 g of CHO/kg body weight, which may have been sufficient to maintain the blood glucose concentration in the placebo group therefore reducing the stimulation of the HPA axis. If stimulation of the HPA axis was reduced than the subsequent release of cortisol may have been reduced as well, thus resulting in a similar hormone profile in both groups. Furthermore, this may have been sufficient to negate possible differences in post exercise immune function between the carbohydrate and placebo groups. Another possible explanation of the minimal effect of carbohydrate supplementation on the immune or hormonal response to the rowing exercise may be that the rowing exercise was not of sufficient duration and intensity to fully deplete the glycogen stores in the subjects. According to Hargreaves (1991), a reference man of 70 kg on a mixed diet would store 450 kg of glycogen in his muscle at rest. Lamb and Murray (1988) have suggested that fatigue would not set in until after these stores had been depleted. The subjects in our

investigation rowed at approximately 72% of VO2max for one hour resulting in a carbohydrate utilization of approximately 202.8 ± 48.0 g for the carbohydrate group and 182.6 ± 78.6 g for the placebo group, suggesting that subjects in both groups did not deplete their muscle glycogen stores. This may have negated any possible benefits of the carbohydrate supplementation on attenuating post exercise changes in the immune system. Finally, the strict dietary control for the 3-day period leading up to the 1-hour row in this study was not used in many other studies investigating the effects of carbohydrate and the immune system. Henson et al (2000) and Nieman et al (1999) do not mention any form of dietary control. Some studies asked their subjects to simply eat the same foods and beverages 1 or 2 days before repeated exercise trials with no restriction or suggestions on macronutrient amounts (Bishop et al, 2002, 2001c). Other studies try to compensate for lack of dietary control by providing one standardized meal (adjusted on body weight) the night before an exercise bout (Bishop et al, 2002) or one entire day's dietary intake (not adjusted for body weight) before the exercise bout (Bacurau et al, 2002). A majority of the studies have given the subjects a list of readily available foods to choose from in order to standardize the amounts of macronutrients consumed for a 2 or 3-day load (Bishop et al, 2001a,b, Henson et al, 1999, Gleeson et al, 1998, Mitchell et al, 1998). However, these studies still require interpretation by the subjects in food selection, where as in this investigation, all subjects diet were modified based on a 3-day dietary record completed by the subject. This allowed the subject to consume food and beverages that they normal eat with only modifications made to the amounts to ensure 55% of total intake from carbohydrate. The combination of the dietary control and pre-exercise meal in this investigation may have been sufficient to minimize

the effects of carbohydrate supplementation on the post exercise immune and hormonal responses. Furthermore, these results may suggest that proper daily and pre-exercise nutrition are sufficient to attenuating the post exercise alterations in hormone concentrations and immune cell counts and function.

3.5 Limitations

Due to the complex interactions of various aspects of the immune system, as well as the effects of the endocrine system on immune function (Gleeson and Bishop, 2000), it is very difficult to account for all effects of exercise on immune function. Other immune parameters or hormone concentrations then those measured may have been affected by the rowing exercise or the carbohydrate supplementation or both. However, the measurements that were taken, were chosen to represent those which most effect or contribute to immune function immediately following exercise and those that may have been effected by carbohydrate supplementation. Also, the immune parameters that were measured were only taken for the first hour of recovery after exercise. Previous research has indicated that some immune parameters take much longer than this time frame to return to resting values (Mackinnon, 1999).

Furthermore, the self-report of cold or upper respiratory tract infections (URTIs) may not have adequately accounted for incidence of illness in the subjects, as it was their own responsibility to judge an illness based on criteria on a cold/URTI questionnaire. A clinical assessment of illness would have been a more accurate gauge of the actually incidence of illness in the subjects. Also, the number of subjects may not have provided sufficient power to determine the incidence of illness in such a short period of time (14 days).

Also, the intensity of the rowing exercise was self-selected by the subject. The intensity of exercise has been directly linked to the rate of carbohydrate utilization (Lamb and Murray, 1988) and the post exercise immune response (Nieman and Pedersen, 2002). Therefore, differences between subjects' work rate during exercise may have elicited different immune or endocrine responses regardless of whether or not the subject was receiving the carbohydrate supplementation or not. Ensuring all subjects had rowed uninterrupted for an hour at least once in the week prior to their actual 1-hour row test minimized the limitation of subject motivation.

Another limitation of this investigation is the duration of the rowing exercise. While the 1-hour row did result in a higher blood glucose concentration immediately following exercise in the CHO group, the exercise bout was not long enough to deplete the muscle glycogen stores of the subjects in the placebo group. An exercise bout of longer duration would have created a greater difference in carbohydrate availability between groups and therefore possibly showing a greater impact of carbohydrate on post exercise immune function. However, the bout of exercise would then not have been applicable to a typical off-season training session for a rower.

3.6 Conclusions

This investigation established that certain immune cell counts and functions were altered for at least an hour following 1 hour of rowing exercise at approximately 72% of VO_2max. Of the immune parameters measured, carbohydrate supplementation was only effective in preventing the rise in circulating lymphocytes and the concentration of $CD3^-/CD16^+$ cells (monocytes and NK cells) immediately following exercise (POST 5), when compared to placebo ingestion. All other blood, immune, and hormone measures were

not significantly different between the carbohydrate and placebo groups. The incidence of illness was not significantly different between the carbohydrate and placebo groups for the 14 days following the 1-hour row. The ineffectiveness of carbohydrate supplementation in attenuating a majority of alterations in post exercise immune measures may be directly related to the intensity and duration of the rowing exercise in this investigation. Furthermore, the lack of significant effects of carbohydrate supplementation on post exercise alterations in the immune response may be due to the fact that all subjects in our investigation received a pre-event meal.

Future research should attempt to more firmly establish the ability of carbohydrate supplementation to attenuate post exercise immune function following various exercise bouts. The inability to draw clear conclusion on the effects of carbohydrate supplementation on immune function following exercise of varying intensities and durations illustrates this need for further research in this area. Future studies should attempt to regulate pre-exercise conditions (activity and diet prior to testing, timing of beverage intake, etc), methodological (adequate time and intensity to deplete carbohydrate stores), and measurement techniques as strictly as possible to more clearly establish a relationship between intensity, duration, and carbohydrate supplementation on post exercise immune function.

Furthermore, research should place more focus on attempting to determine the clinical significance (i.e. Increased incidence of illness), if any, of these statistically significant alterations in the immune system following exercise.

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Table 3-1. Monoclonal antibody combinations used for lymphocyte phenotyping.

WELL	mAbs	Primary Cells Identified
1	None (Cells only)	Spontaneous Binding (Background)
2	CD3-B/CD4-FITC/CD8-PE	$CD3^+$ (T cells) $CD4^+$ (T_H cells) $CD8^+$ (T_C or T_S cells)
3	CD4-FITC/CD8-B/CD25-PE	$CD25^+$ (IL-2 receptor α subunit expressed on activated NK, B cells, and monocytes) $CD4^+/CD25^+$ (activated T_H cells) $CD8^+/CD25^+$ (activated T_C or T_S cells)
4	CD3-FITC/CD16-PE/CD56-B	$CD16^+$ (NK cells, monocytes) $CD3^-/16^+/CD56^+$ (NK cells)
5	CD14-PE	$CD14^+$ (monocytes)
6	CD20-FITC	$CD20^+$ (B cells)

Table 3-2. Subject characteristics and performance measurements. Values are means ± SD.

	PLA	CHO
Age (years)	26.6 ± 6.7	30.0 ± 8.1
Height (cm)	180.4 ± 5.9	179.3 ± 9.8
Weight (kg)	75.8 ± 7.8	77.3 ± 7.5
VO_2max/VT Test		
VO_2 (l/min)	4.133 ± 0.545	4.154 ± 0.463
HR_{max} (bpm)	187 ± 12	190 ± 11
Maximum Power Output (W)	359.1 ± 62.3	364.3 ± 61.6
2000m Simulated Rowing Race		
2000m Time (sec)	427.5 ± 18.1	427.2 ± 21.4
HR_{max} (bpm)	186 ± 12	188 ± 11
Mean Power Output (W)	287.6 ± 36.2	291.3 ± 43.5
MRM Testing		
Predicted 1RM Bench Press (kg)	81.6 ± 15.6	80.6 ± 17.4
Predicted 1RM Leg Press (kg)	284.1 ± 81.3	309.6 ± 45.7

Table 3-3. 1-Hour row performance and physiological measurements. Values are means ± SD.

	PLA	CHO
Distance (m)	14733 ± 839	14506 ± 906
Average Power Output	193.6 ± 32.2	185.1 ± 35.3
Average 500m Split Time (min:sec)	2:01.2 ± 0:07.0	2:04.3 ± 0.078
Average Heart Rate	175 ± 13	175 ± 11
Average VO_2 (L/min)	3.036 ± 0.533	2.914 ± 0.402
Average % of VO_2max	73.2 ± 4.0	70.2 ± 5.4
Average RER	0.97 ± 0.04	0.99 ± 0.06

Table 3-4. Estimated fuel utilization during the 1-hour row. Values are means ± SD.

	PLA	CHO
Energy Expended (kcal)	911.2 ± 168.6	877.4 ± 125.6
Kcal from Carbohydrate (%)	87.0 ± 11.7	91.2 ± 12.8
Kcal from Carbohydrate (kcal/min)	13.4 ± 3.5	13.5 ± 3.2
Carbohydrate Utilization (g of CHO/min)	3.4 ± 0.9	3.4 ± 0.8
Kcal from Fat (%)	13.0 ± 11.7	8.8 ± 12.8
Kcal from Fat (kcal/min)	1.7 ± 1.8	1.1 ± 1.5
Fat Utilization (g of Fat/min)	0.19 ± 0.19	0.12 ± 0.17

Table 3-5. Mean values of the 3-day dietary intake prior to the 1-hour row. Values are means ± SD.

	PLA	CHO
Carbohydrate (%)	55 ± 1	55 ± 1
Fat (%)	25 ± 5	28 ± 4
Protein (%)	18 ± 5	16 ± 3
Total Calories (kcal)	3045 ± 579	2572 ± 924

Table 3-6. Natural killer cell cytotoxic activity (expressed as % specific lysis). Values are means ± SD.

Effector:Target Cell Ratio	GROUP	REST	POST 5	POST 60
3.125:1	PLA	9.3 ± 7.2	10.9 ± 6.3	12.6 ± 19.5
	CHO	11.1 ± 9.8	12.0 ± 12.9	5.9 ± 8.6
6.25:1	PLA	31.5 ± 12.8	44.5 ± 14.3	19.9 ± 14.0*†
	CHO	29.7 ± 20.8	28.5 ± 19.3	19.7 ± 13.0*†
12.5:1	PLA	58.3 ± 9.9	51.3 ± 9.0	34.5 ± 13.5*†
	CHO	49.1 ± 18.7	48.1 ± 15.6	37.5 ± 9.7*†
25:1	PLA	54.3 ± 9.9	55.6 ± 11.3	47.5 ± 11.4†
	CHO	57.4 ± 9.6	54.5 ± 14.5	51.7 ± 12.8†
50:1	PLA	65 ± 10.7	63.7 ± 11.2	67.2 ± 12.4
	CHO	70.6 ± 9.6	65.2 ± 13.2	65.8 ± 16.3

* Significantly different than REST value ($p<0.05$).
† Significantly different than POST 5 value ($p<0.05$).

Table 3-7. Natural killer cell cytotoxic activity (expressed in lytic units (L.U. × 10^{-3} cells)). Values are means ± SD.

	PLA	CHO
REST	7.68 ± 5.49	6.88 ± 5.99
POST 5	10.94 ± 8.56*	13.81 ± 12.88*
POST 60	7.55 ± 4.91†	6.43 ± 2.49†

* Significantly different than REST value ($p<0.05$).
† Significantly different than POST 5 value ($p<0.05$).

Table 3-8. Lymphocyte phenotyping data (expressed as the percent of gated cells in sample). Values are means ± SD.

		REST	POST 5	POST 60
WELL 1				
$CD3^+$	**PLA**	67.0 ± 23.0	61.4 ± 10.5	71.6 ± 11.1
	CHO	68.2 ± 20.5	66.9 ± 20.6	69.7 ± 23.4
$CD3^+/CD4^+$	**PLA**	43.4 ± 17.1	32.4 ± 11.1	46.4 ± 8.8
	CHO	43.1 ± 18.0	38.1 ± 13.1	42.7 ± 22.8
$CD3^+/CD8^+$	**PLA**	31.1 ± 16.9	30.5 ± 16.6	37.6 ± 19.9
	CHO	26.2 ± 19.9	29.3 ± 15.9	27.4 ± 19.1
WELL 2				
$CD25^+$	**PLA**	14.2 ± 8.6	11.2 ± 6.6	12.9 ± 6.8
	CHO	11.0 ± 7.7	10.5 ± 9.1	15.4 ± 12.2
$CD4^+/CD25^+$	**PLA**	8.9 ± 5.7	6.6 ± 4.5	8.6 ± 4.9
	CHO	5.2 ± 2.8	6.7 ± 4.7	7.7 ± 4.7
$CD8^+/CD25^+$	**PLA**	4.1 ± 4.7	2.2 ± 2.6	1.9 ± 2.2
	CHO	2.4 ± 4.2	1.5 ± 1.8	10.0 ± 19.0
WELL 3				
$CD3^-/CD16^+$	**PLA**	14.1 ± 9.6	23.1 ± 12.5*‡	10.4 ± 6.7†
	CHO	9.7 ± 8.5	13.0 ± 12.9	9.1 ± 10.8†
$CD3^-/CD56^+$	**PLA**	8.5 ± 7.7	17.7 ± 13.7*	9.1 ± 9.6†
	CHO	6.9 ± 7.6	10.6 ± 8.5*	9.6 ± 10.4†
$CD3^-/CD16^+/CD56^+$	**PLA**	8.4 ± 7.0	17.5 ± 13.2*	7.4 ± 6.2†
	CHO	8.6 ± 4.4	13.3 ± 6.1*	7.2 ± 4.7†
WELL 4				
$CD14^+$	**PLA**	3.8 ± 2.9	4.1 ± 3.6	7.0 ± 4.8*†
	CHO	4.1 ± 4.4	4.8 ± 5.1	10.9 ± 10.2*†
WELL 5				
$CD20^+$	**PLA**	7.4 ± 4.1	9.9 ± 3.9	10.2 ± 5.6*
	CHO	9.9 ± 6.3	8.7 ± 1.9	11.2 ± 4.5*

* Significantly different than REST value ($p<0.05$). † Significantly different than POST 5 value ($p<0.05$). ‡ Significantly different than CHO group at POST 5 ($p<0.05$).

Table 3-9. Lymphocyte Concentrations (cells ($\times 10^9$) /L). Values are means ± SD.

		REST	POST 5	POST 60
WELL 1				
$CD3^+$	**PLA**	1.41 ± 0.57	1.79 ± 0.38	0.79 ± 0.28*†
	CHO	1.46 ± 0.82	1.36 ± 0.50	0.78 ± 0.39*†
$CD3^+/CD4^+$	**PLA**	0.94 ± 0.42	0.92 ± 0.31	0.53 ± 0.23*†
	CHO	0.90 ± 0.65	0.78 ± 0.33	0.45 ± 0.29*†
$CD3^+/CD8^+$	**PLA**	0.65 ± 0.31	0.87 ± 0.46	0.41 ± 0.20*†
	CHO	0.58 ± 0.42	0.60 ± 0.38	0.33 ± 0.35*†
WELL 2				
$CD25^+$	**PLA**	0.28 ± 0.13	0.34 ± 0.21	0.14 ± 0.08*†
	CHO	0.22 ± 0.15	0.19 ± 0.10	0.19 ± 0.21*†
$CD4^+/CD25^+$	**PLA**	0.18 ± 0.09	0.19 ± 0.13	0.10 ± 0.06*†
	CHO	0.11 ± 0.09	0.12 ± 0.06	0.09 ± 0.09*†
$CD8^+/CD25^+$	**PLA**	0.08 ± 0.08	0.07 ± 0.08	0.02 ± 0.02
	CHO	0.04 ± 0.08	0.02 ± 0.02	0.13 ± 0.24
WELL 3				
$CD3^-/CD16^+$	**PLA**	0.30 ± 0.22	0.67 ± 0.36*‡	0.12 ± 0.08*†
	CHO	0.21 ± 0.20	0.32 ± 0.38	0.08 ± 0.70*†
$CD3^-/CD56^+$	**PLA**	0.19 ± 0.19	0.51 ± 0.40*	0.10 ± 0.09†
	CHO	0.14 ± 0.17	0.23 ± 0.19*	0.08 ± 0.08†
$CD3^-/CD16^+/CD56^+$	**PLA**	0.18 ± 0.17	0.51 ± 0.40*	0.08 ± 0.06†
	CHO	0.18 ± 0.14	0.29 ± 0.19*	0.07 ± 0.04†
WELL 5				
$CD20^+$	**PLA**	0.15 ± 0.08	0.26 ± 0.08*	0.10 ± 0.06*†
	CHO	0.21 ± 0.15	0.19 ± 0.07*	0.11 ± 0.05*†

* Significantly different than REST value ($p<0.05$). † Significantly different than POST 5 value ($p\leq0.05$). ‡ Significantly different than CHO group at POST 5 ($p<0.05$).

Table 3-10. CD4 to CD8 Ratio ($CD3^+/4^+$ cells divided by $CD3^+/8^+$ cells). Values are means ± SD.

	PLA	CHO
REST	1.6 ± 0.9	1.7 ± 0.9
POST 5	1.3 ± 0.7	1.6 ± 0.8
POST 60	1.7 ± 1.1	2.1 ± 2.1

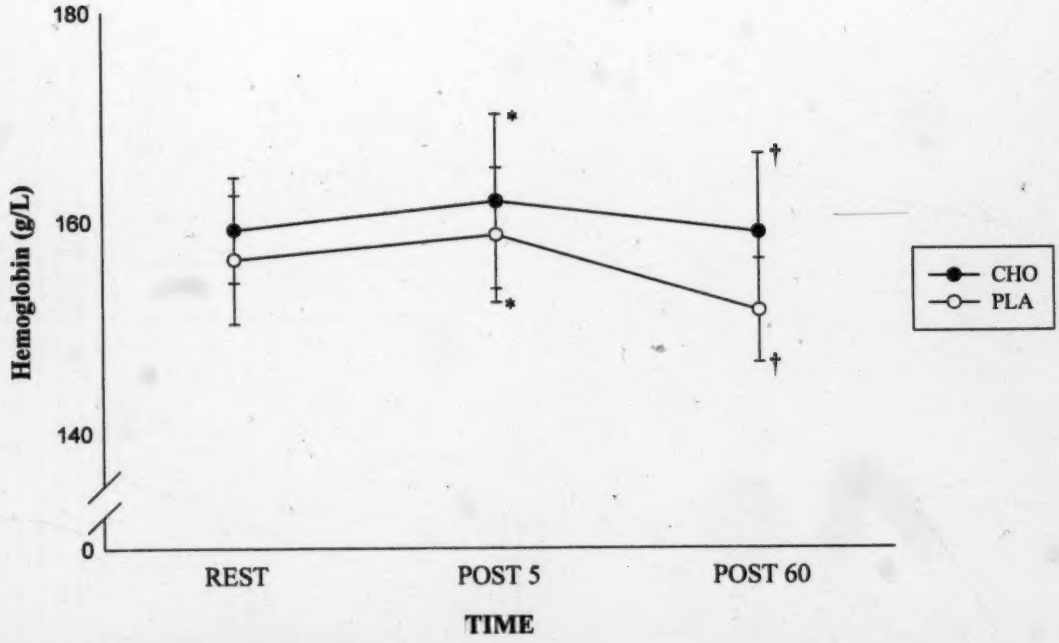

Figure 3-1. Hemoglobin concentrations at rest and following 1 hour of rowing exercise. (Error bars are = ± SD)

* Significantly different than REST value ($p<0.05$)

† Significantly different than POST 5 value ($p<0.05$)

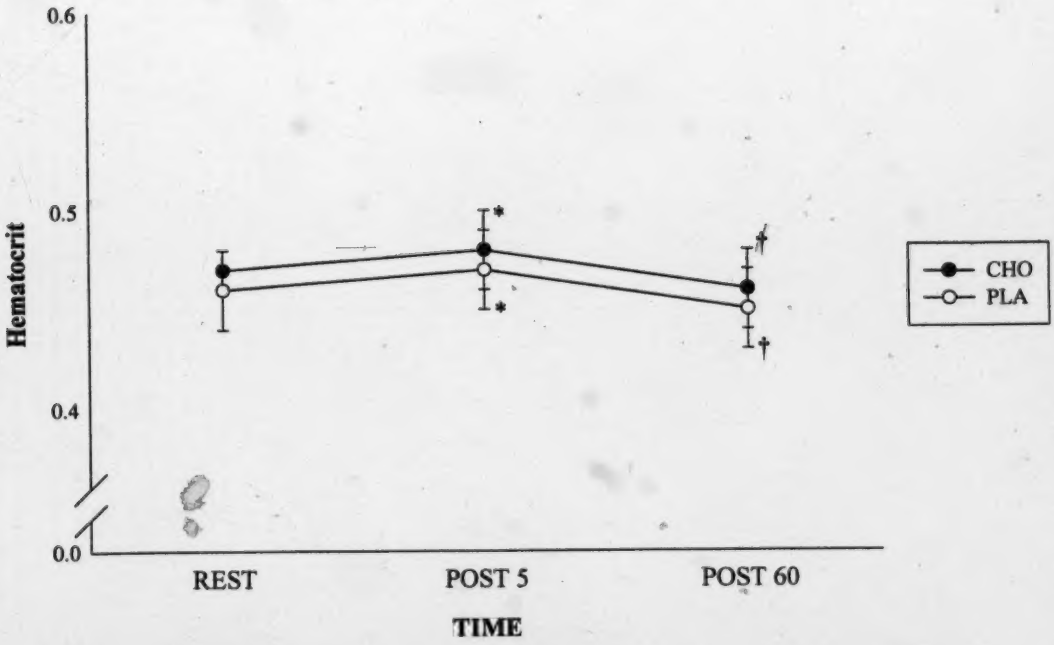

Figure 3-2. Hematocrit levels at rest and following 1 hour of rowing exercise. (Error bars are = ± SD)

* Significantly different than REST value ($p<0.05$)

† Significantly different than POST 5 value ($p<0.05$)

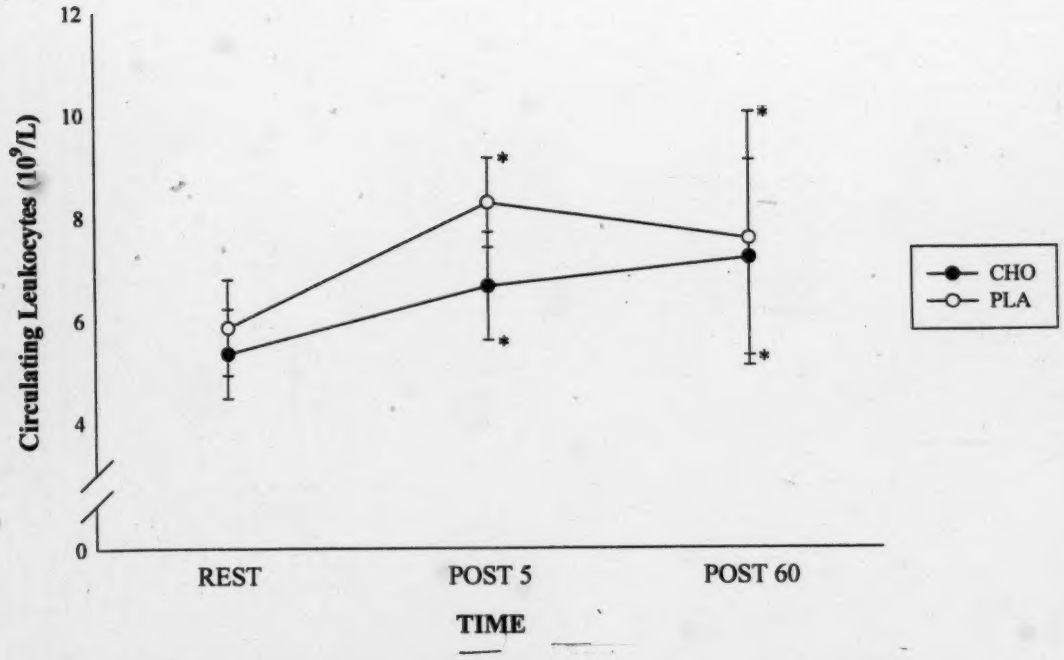

Figure 3-3. Circulating leukocytes ($\times 10^9$ cells/L) at rest and following 1 hour of rowing exercise. (Error bars are = ± SD)
* Significantly different than REST value ($p<0.05$)

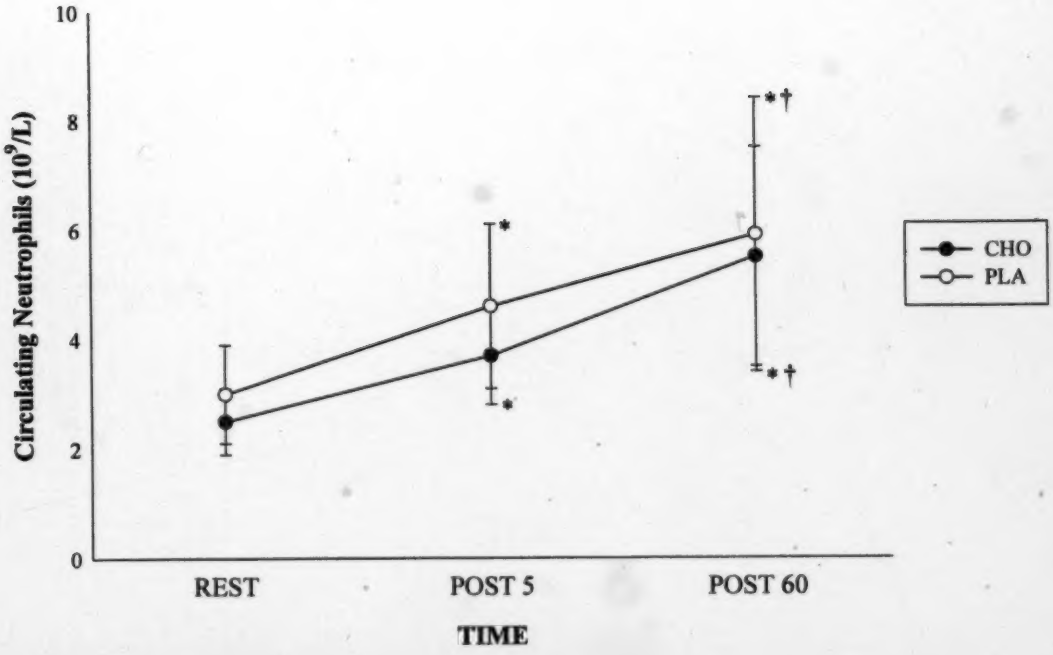

Figure 3-4. Circulating neutrophils (× 10^9 cells/L) at rest and following 1 hour of rowing exercise. (Error bars are = ± SD)

* Significantly different than REST value ($p<0.05$)

† Significantly different than POST 5 value ($p<0.05$)

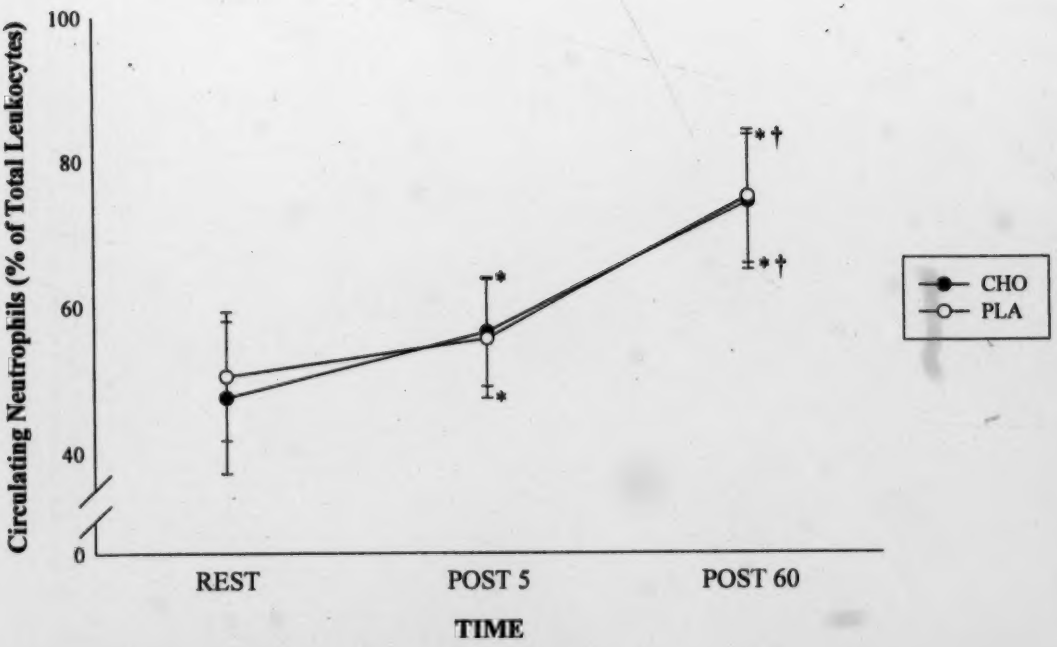

Figure 3-5. Circulating neutrophils expressed as a % of total circulating leukocytes. (Error bars are = ± SD)

* Significantly different than REST value ($p<0.05$)

† Significantly different than POST 5 value ($p<0.05$)

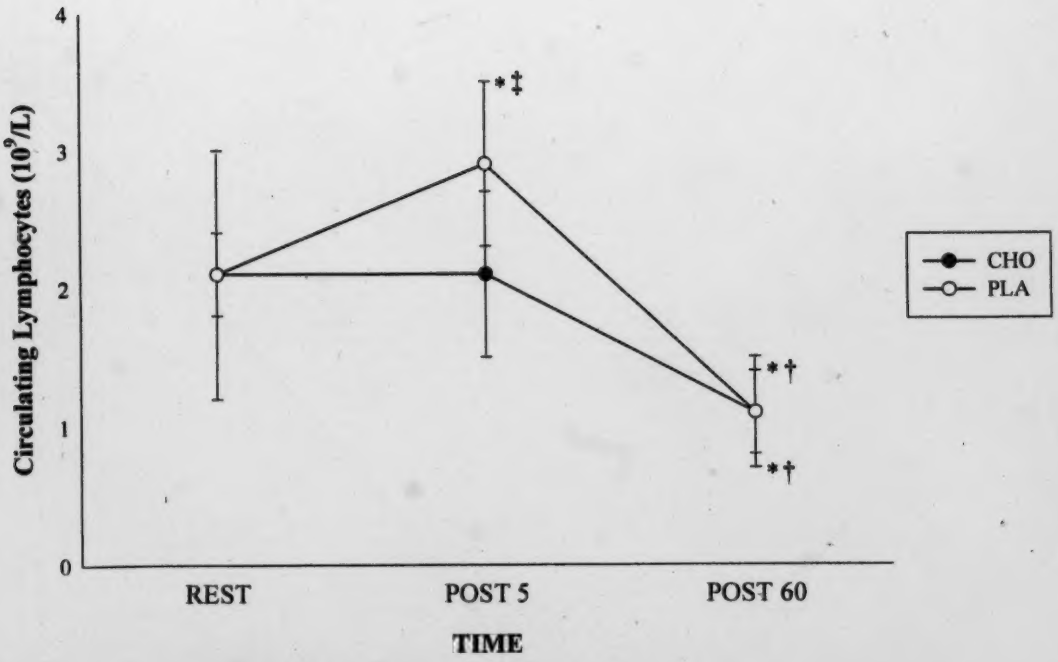

Figure 3-6. Circulating lymphocytes (× 10^9 cells/L) at rest and following 1 hour of rowing exercise. (Error bars are = ± SD)

* Significantly different than REST value ($p<0.05$)
† Significantly different than POST 5 value ($p<0.05$)
‡ Significantly different than CHO group at POST 5 ($p<0.05$)

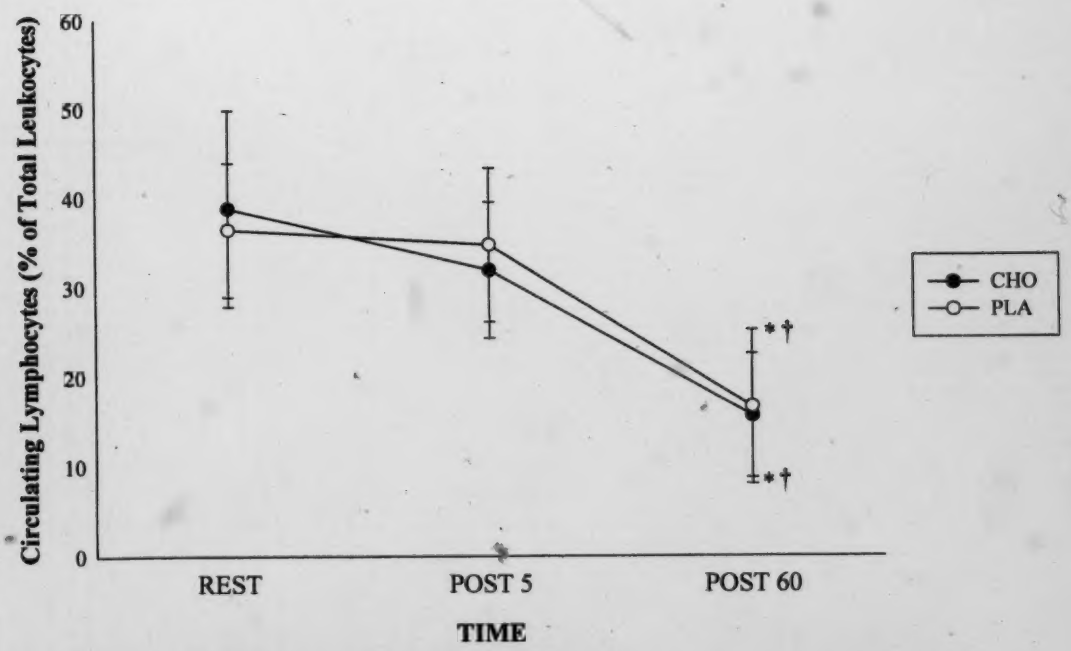

Figure 3-7. Circulating lymphocytes expressed as a % of total circulating leukocytes at rest and following 1 hour of rowing exercise. (Error bars are = ± SD)
* Significantly different than REST value ($p<0.05$)
† Significantly different than POST 5 value ($p<0.05$)

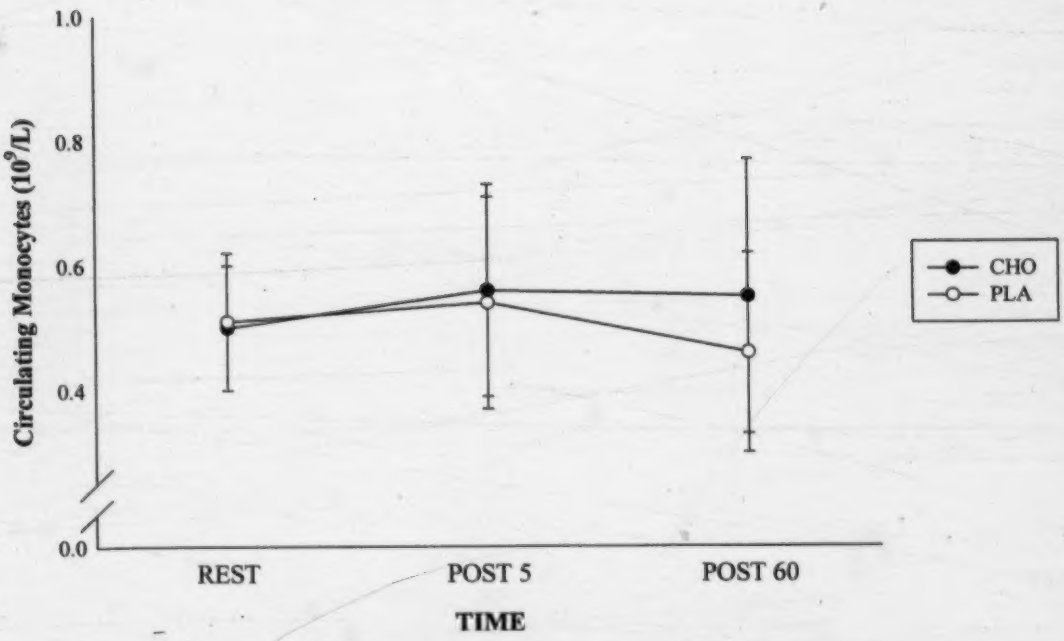

Figure 3-8. Circulating monocytes ($\times 10^9$ cells/L) at rest and following 1 hour of rowing exercise. (Error bars are = ± SD)

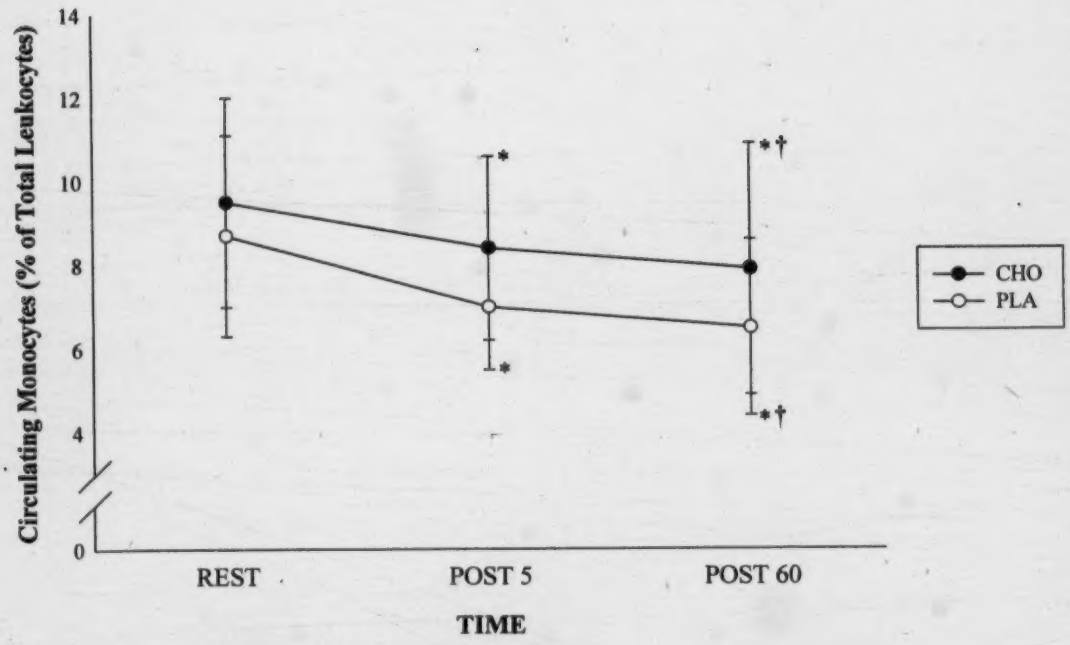

Figure 3-9. Circulating monocytes expressed as a % of total circulating leukocytes at rest and following 1 hour of rowing exercise. (Error bars are = ± SD)
* Significantly different than REST value ($p<0.05$)
† Significantly different than POST 5 value ($p<0.05$)

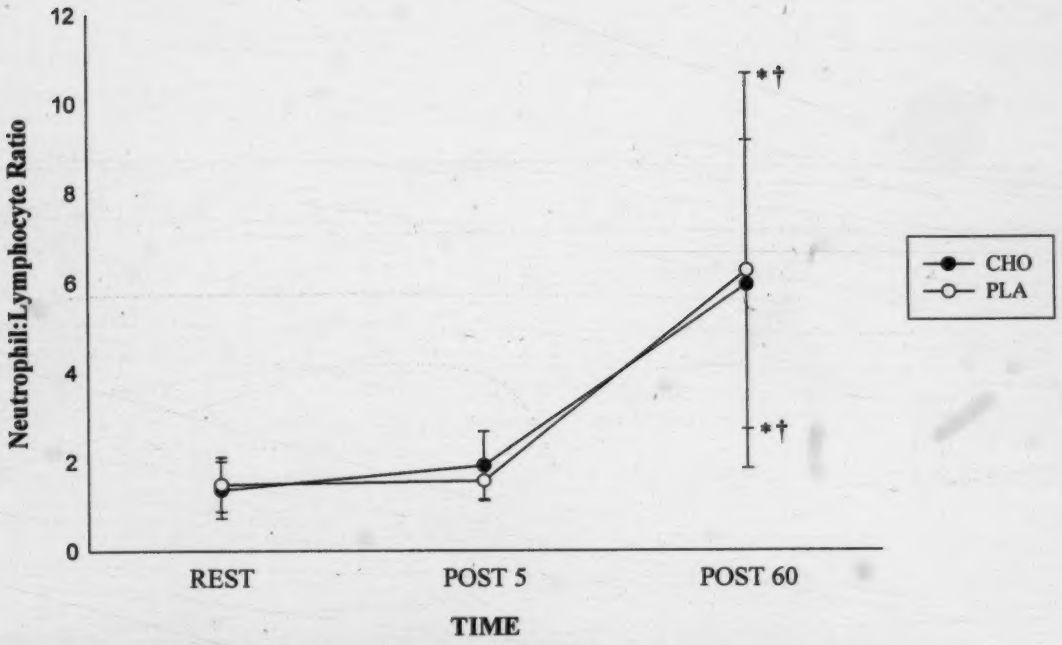

Figure 3-10. Neutrophil:Lymphocyte Ratio (Circulating Neutrophils ($\times 10^9$ cells/L)/Circulating Lymphocytes ($\times 10^9$ cells/L)) at rest and following 1 hour of rowing exercise. (Error bars are = ± SD)

* Significantly different than REST value ($p<0.05$)

† Significantly different than POST 5 value ($p<0.05$)

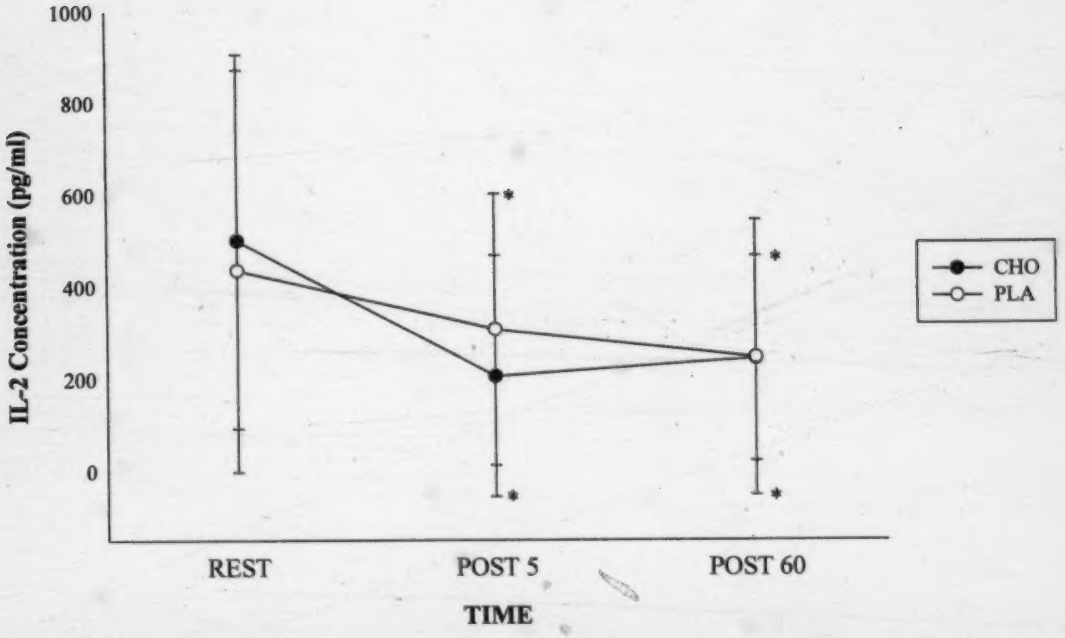

Figure 3-11. IL-2 concentrations following 48 hours of PHA stimulation on isolated lymphocyte samples taken at rest and following 1 hour of rowing exercise. (Error bars are = ± SD)

* Significantly different than REST value ($p<0.05$)

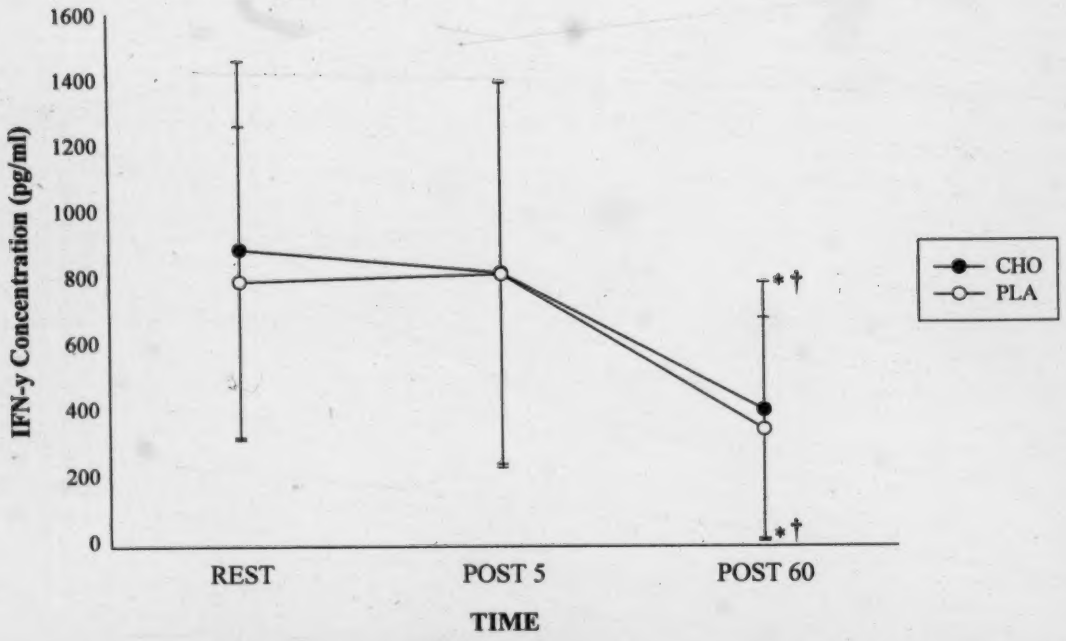

Figure 3-12. IFNγ concentrations following 48 hours of PHA stimulation on isolated lymphocyte samples taken at REST and following 1 hour of rowing exercise. (Error bars are = ± SD)

* Significantly different than REST value ($p<0.05$)

† Significantly different than POST 5 value ($p<0.05$)

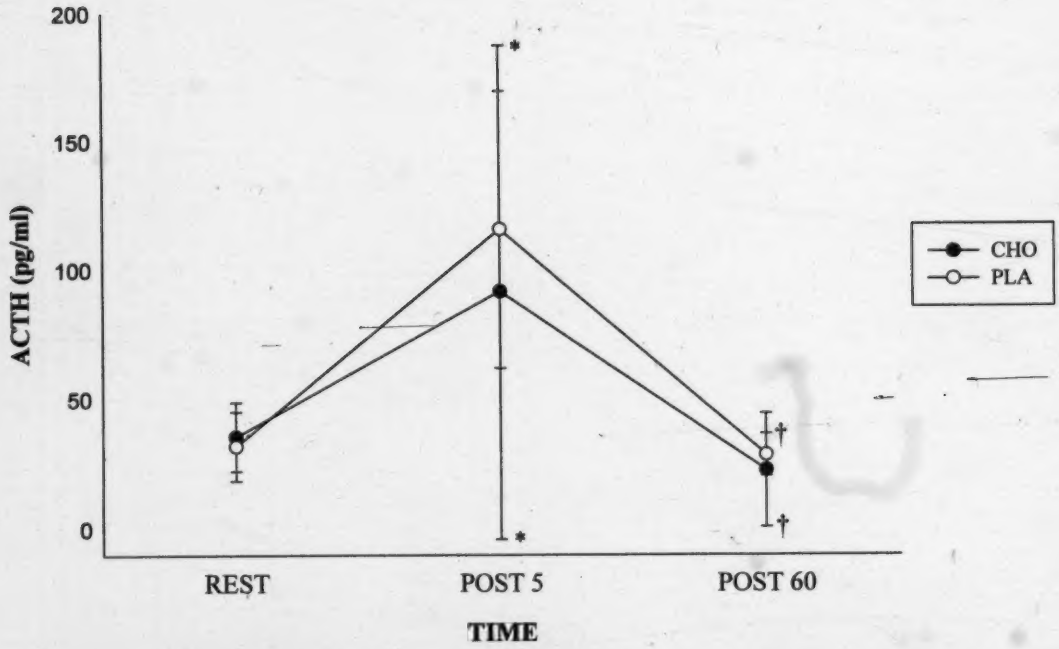

Figure 3-13. Circulating plasma ACTH concentrations at rest and following 1 hour of rowing exercise. (Error bars are = ± SD)

* Significantly different than REST value ($p<0.05$)

† Significantly different than POST 5 value ($p<0.05$)

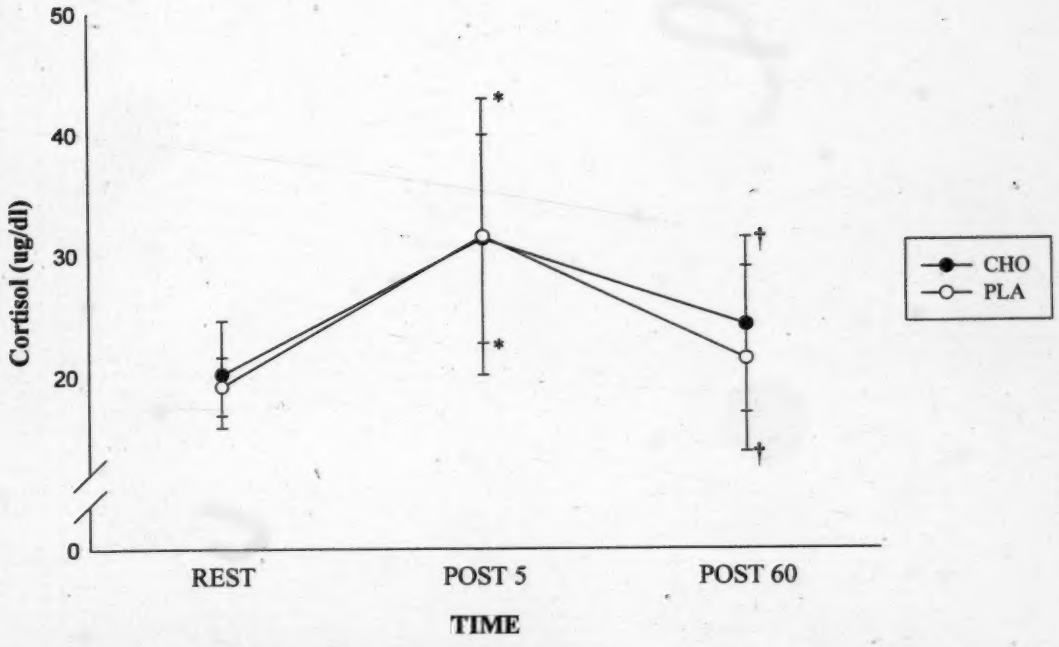

Figure 3-14. Circulating plasma cortisol concentrations at rest and following 1 hour of rowing exercise. (Error bars are = ± SD)

* Significantly different than REST value ($p<0.05$)

† Significantly different than POST 5 value ($p<0.05$)

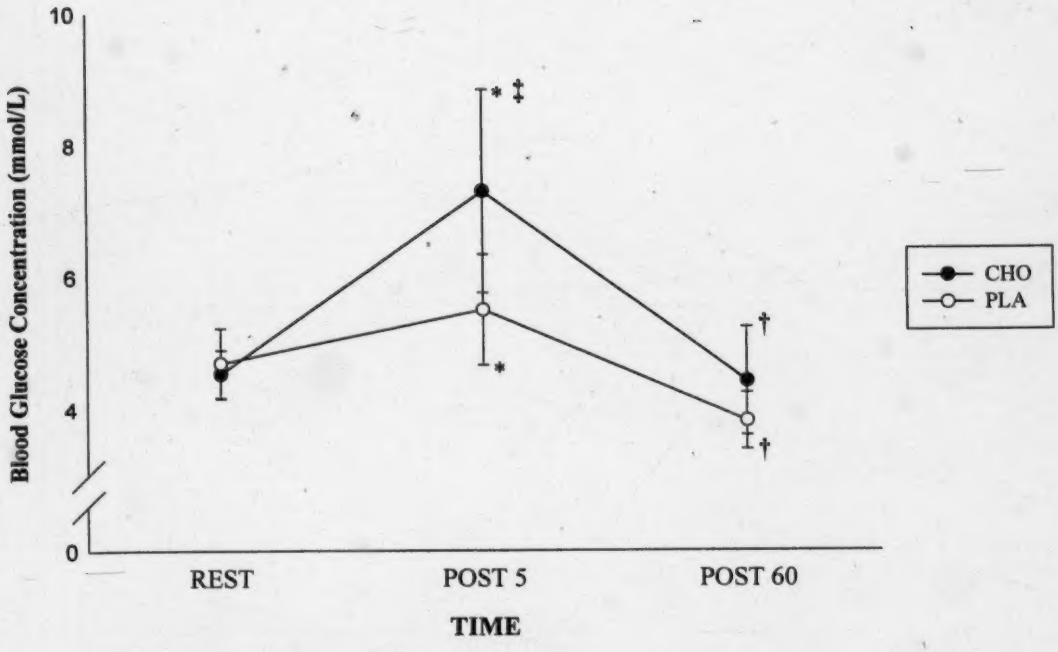

Figure 3-15. Plasma glucose concentrations at rest and following 1 hour of rowing exercise. (Error bars are = ± SD)

* Significantly different than REST value ($p<0.05$)

† Significantly different than POST 5 value ($p<0.05$)

‡ Significantly different than CHO group at POST 5 ($p<0.05$)

CHAPTER 4

GENERAL DISCUSSION AND CONCLUSIONS

4.1 Discussion

Following intense prolonged exercise an individual may be at risk for infection due to transient alterations in post exercise immune function (Nieman and Pedersen, 2002). These alterations may provide an "open window" for infections to take hold in the body (Nieman, 1994). One suggested cause of this temporary impairment in immune function is caused by the stimulation of the Hypothalamus-Pituitary-Adrenal (HPA) axis, which results in the subsequent release of stress hormones (Gleeson and Bishop, 2000). High levels of stress hormones have been linked to impairment of certain aspects of immunity (Nieman and Pedersen, 2002). Supplementing an individual with carbohydrate before, during, and after exercise has been proposed as a means of attenuating the changes in the immune system following strenuous exercise (Nieman and Pedersen, 2002, Gleeson and Bishop, 2000). The suggested mechanism is the maintenance of blood glucose, in order to both reduce the stimulation of the HPA axis and the subsequent release of adrenocorticotrophic (ACTH) and cortisol, and to provide adequate metabolic fuel for immune cell metabolism, activity, and proliferation (Gleeson and Bishop, 2000).

The nature of rowing exercise provides a good model to study post exercise immune function. Rowing requires a large majority of the body's muscle mass and utilizes all three of the body's energy systems: the ATP-PC, anaerobic glycolysis, and aerobic energy system (Steinacker et al, 1998). These characteristics of rowing exercise place a high level of physiological stress on the body and results in high levels of stress hormones (Secher, 1993). As stated above, high levels of stress hormones may causes

changes in the immune response, leaving a possibility of an "open window" to infection (Shephard, 1998). This is supported by Castell et al (1996) who have suggested that upper respiratory tract infections (URTIs) are more prevalent in rowing than in other athletic groups, including marathoners and cross country runners. Therefore, intense prolonged rowing exercise would provide a suitable model to induce post exercise immune changes, and furthermore, provides an opportunity to examine the effects of carbohydrate supplementation on these post exercise alterations in immune function.

The immune response to short duration, high intensity (Nielsen, 1996) and long duration low intensity rowing (Nieman, 1999) has been previously explored. The immune response to prolonged, moderate intensity rowing has not been explored and would mimic an off-season training session for a rower. It was hypothesized that 1 hour of rowing exercise would alter the acute immune response following exercise, when compared to values taken at rest. Furthermore, it was hypothesized that carbohydrate supplementation before, during, and after exercise would attenuate these changes.

The results from this investigation suggest that 1 hour of rowing exercise at ≈ 72% of VO_2max provides a significant physiological stress to cause changes in post exercise immune function when compared to resting levels. These results are supported by previous research (Mackinnon, 1999), although the changes reported in the current investigation were not as great as those seen in the current literature. The main reasons for this discrepancy is that the duration or intensity, or the combination of both, was not as great as many of the exercise bouts reported in the literature.

While the measures taken following exercise clearly indicate alterations in immune function following exercise, the effect of carbohydrate supplementation on these

changes was minimal. The only immune measures that were attenuated by carbohydrate ingestion when compared to the placebo group were the increases in circulating lymphocytes and $CD3^-/CD16^+$ cells (monocytes and NK cells) immediately following exercise. All other immune, hormonal, or blood parameters that were measured were not significantly influenced by carbohydrate supplementation despite higher blood glucose concentrations following exercise. These results are supported by Bishop et al (2001) who found no changes in immune or hormonal measures following cycling exercise, with carbohydrate supplementation, at 75% of VO_2max to fatigue. However, other studies of similar intensities and duration have found equivocal results. Bacurau et al (2002), Mitchell et al (1998), and Gleeson et al (1998) all found significant effects of carbohydrate on post exercise immune function following cycling exercise ranging from 70-75% of VO2max for 60 to 80 minutes. The only major difference between these three studies and the present investigation is that all subjects in the current study were given a pre-exercise meal consisting of 1 g of CHO/kg of body weight, regardless of their experimental group. This may have maintained the blood glucose concentrations in the placebo group enough to allow these subjects to row at a similar intensity to the rowers in the carbohydrate group, as well as prevent any greater stimulation of the HPA axis. If this is the case, in combination with the fact that post exercise immune function is related to exercise intensity and duration (Nieman and Pedersen, 2002), then the subjects in both groups in this investigation may have had very similar alterations in immune function following the rowing exercise, regardless of carbohydrate intake. This may suggest that pre-exercise nutrition status is more important than carbohydrate supplementation during and after exercise in attenuating post exercise immune changes.

4.2 Limitations

Due to the vast parameters that can be measured as indicators of post exercise immune function, it would be impossible and impractical to measure all of them. However, this does leave the possibility of missing an important immune response to exercise, carbohydrate, or both. The variables that were measured were chosen based on their importance to the immune response immediately following exercise as well as the possible beneficial effects that carbohydrate may have on them. Also, due to many constraints, the changes were only tracked for the first hour following exercise. Many previous studies have reported continued changes outside of this time frame.

The motivation of the subjects can definitely be a limiting factor when subjects are allowed to row at a self-selected pace. In addition, since the immune response to exercise and rates of carbohydrate utilization are directly related to the intensity of exercise, the intensity that the rower chose to row at affected both the post exercise immune measures and the possible effects of carbohydrate on these measures. In order to minimize this factor, all subjects had completed at least one 1-hour row in the week prior to their actual test.

In attempt to make the results of this investigation applicable to an actual off-season training session, we may have limited the possible effect of carbohydrate on alterations in immune parameters following exercise. The lack of significant effects of carbohydrate supplementation on immune, blood, and hormonal parameters may be due to the fact that muscle glycogen stores were not significantly depleted in subjects in the placebo group, and therefore supplementation did not make a significant difference.

However, the results of this study may provide useful guidelines for actual rowing training sessions.

4.3 Conclusions

One hour of rowing exercise at ≈ 72% of VO_2max is a sufficient stimulus to causes temporary changes in immune function following exercise. Carbohydrate supplementation before, during, and after exercise appears to have minimal effects on the post exercise immune and hormonal responses. The results of this investigation suggest that proper dietary intake and pre-exercise nutrition may be more important in reducing the extent of exercise induced alteration in immune function, and in turn reducing the risk of contracting an illness than supplementation during exercise.

Future research should focus on establishing a clear relationship (if one exists) between carbohydrate supplementation and the intensity and duration of the exercise bout. Also, more information is needed to determine if any of these changes are actually increasing the risk of illness in individuals following prolonged or strenuous exercise.

4.4 References

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APPENDIX A

PRE-EXERCISE MEAL & EXPERIMENTAL BEVERAGE NUTRITIONAL INFORMATION

CLIF® Bar Nutritional Information

	Average of All Types
Mass (g)	67.45 ± 1.81
Carbohydrate (g)	45.35 ± 4.43
Protein (g)	8.06 ± 2.88
Fat (g)	3.62 ±1.29
Total kcals	246.36 ± 9.24
Kcal/g	3.65 ± 0.06

≈ Glycemic Load/Index = 101 ± 27 (Reference Food: Glycemic Load/Index of Glucose = 100)

Experimental Beverage Nutritional Information

	PLA	CHO
Polycose ®		
Amount (g/kg of body weight)	–	1
Energy (kcal/g)	–	3.8
Crystal Light ®		
Amount of Carbohydrate (g/L)	1.6	1.6
Energy (kcal/L)	16	16

≈ Glycemic Load/Index (for Gatorade®) = 100 ± 21 (Reference Food: Glycemic Load/Index of Glucose = 100)

Glycemic Indexes obtained from:

Gretebeck, R., Gretebeck, K., Tittelbach, T. (2002). Glycemic index of popular sport drinks and energy foods. *Journal of the American Dietetic Association.* 102(3): 415-417.

APPENDIX B

SAMPLE DIETARY INTAKE RECORD

	Menu Item		Unit of Measure	Number of Units	Description of Menu Item		
	Enter all foods, beverages, etc. consumed as menu items. For every menu item, include Toppings or additives added to the menu item at the time of eating.		Enter the word: "cup", "ounce", "number", "teaspoon" etc…		Brand	Type Of Flavour	Method of Cooking
	Menu Item						
	Toppings or Additives						
M	Menu Item						
O	Toppings or Additives						
R							
N	Menu Item						
I	Toppings or Additives						
N							
G	Menu Item						
	Toppings or Additives						
M	Menu Item						
E	Toppings or Additives						
A							
L	Menu Item						
	Toppings or Additives						
	Mark (x) One Category	Eaten at your home					
		Eaten away from your home					
		Did not eat					

Day One

	Menu Item		Unit of Measure		Description of Menu Item		
	Enter all foods, beverages, etc. consumed as menu items. For every menu item, include any Toppings or additives added to the menu item at the time of eating.		Enter the word: "cup", "ounce", "number", "teaspoon" etc…	Number of Units	Brand	Type Of Flavour	Method of Cooking
	Menu Item						
	Toppings or Additives						
M	Menu Item						
O	Toppings or Additives						
R							
N	Menu Item						
I	Toppings or Additives						
N							
G	Menu Item						
	Toppings or Additives						
S	Menu Item						
N	Toppings or Additives						
A							
C	Menu Item						
K	Toppings or Additives						
	Mark (x) One Category	Eaten at your home					
		Eaten away from your home					
		Did not eat					

Day One

APPENDIX C

COLD/URTI ASSESMENT QUESTIONNAIRE & ACCOMPANYING INFORMATION SHEET

Information Sheet

Purpose

The purpose of completing this questionnaire is to provide the investigators with information regarding the incidence of colds or upper respiratory tract infections (URTIs) after the completion of the final 1-hour row. You will fill out the questionnaires (if necessary) for 2 weeks following the 1-hour row. This will be used to compare the rates of illness in the experimental group (those who receive carbohydrate supplementation for the 1-hour row) to the control group (placebo).

Instructions

- You should begin to fill out the questionnaire when you feel a cold/URTI coming on (at the first sign of any symptoms), and continue to fill out the questionnaire each day (add additional days on the back of the questionnaire if necessary) until all symptoms have subsided.
- Follow the instructions given on the questionnaire.
- If you have any questions regarding the completion of the questionnaire, please ask one of the investigators or the supervisor for your training time.
- A separate questionnaire should be filled out for each time that you contract a cold or URTI.

Confidentiality

All the completed questionnaires will be kept confidential and any data analysis will be completed by one of the investigators. The questionnaires will be collected after each training time by the supervisor to ensure that no one else has access to your completed questionnaire.

Cold/URTI Assessment Questionnaire

Please complete the following questionnaire if you begin to feel the symptoms of, or develop a cold or similar upper respiratory tract infection (URTI). Continue to fill out the form according to the instructions given until the symptoms have subsided (add any additional days on the back of the form if required).

Name: _____

Cold/URTI Duration: _____ **days**

Dates: From _____ **to** _____

Symptoms	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Duration*
Runny Nose								
Sneezing								
Watery Eyes								
Stuffy Nose								
Aching Muscles								
Sore Throat								
Headache								
Cough								
Fever								
Chills								
Hoarseness								
Shortness of Breath								
Tiredness								
TOTAL								

* In the **Duration** column, indicate the **number of days** you experienced a specific **symptom** (score between 0 to 7, or higher if the length of the cold/URTI is longer than 7 days)

Score the Symptoms on each day as follows:

Absent	Mild	Moderate	Severe
0	1 2 3	4 5 6	7 8 9
No Symptom	1 = Very Slight Discomfort 2 = Slight Discomfort 3 = Evident Discomfort	4 = Uncomfortable Working 5 = Continuously Tired 6 = Evidently Sick	7 = Absence From Work 8 = Occasionally Bedridden 9 = Bedridden

Was your Cold/URTI (Please circle one of the following):

Less Severe Than Normal **Normal** **More severe than normal**

Please return to the "Completed Questionnaire" envelope after completely filling out.